Biological Effects of Short-Term, High-Concentration Exposure to Methyl Isocyanate. II. Blood Chemistry and Hematologic Evaluations

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Introduction

The objectives of the inhalation studies with methyl isocyanate (MIC) were to investigate the possible causes of death resulting from overexposure to this chemical. The first hypothesis was that exposure to MIC caused a massive inhibition of cholinesterase (ChE) activity. *In vitro* studies described by Brown et al. (1) with toluene diisocyanate and hexamethylene diisocyanate demonstrated an inhibition of plasma ChE activity. These studies suggested that toxic respiratory effects observed following exposure to these chemicals may be explained by ChE inhibition.

A second hypothesis was that death occurred as the result of carbamylation of hemoglobin (Hb). Carbamylation of sickled hemoglobin (Hb S) with cyanates or isocyanates inhibits erythrocyte sickling (2). This inhibition is related to the carbamylation of the beta-chain of Hb S, which precludes the formation of electrostatic bonds that stabilize the deoxygenated conformation of the hemoglobin molecule. It is the formation of these bonds that causes sickling under conditions of low oxygen tension when Hb S is present. Carbamylation stabilizes the oxygenated conformation of Hb S, thus increasing its affinity for oxygen. The increase in affinity is characterized by a leftward shift of the oxygen-dissociation curve and, in the case of Hb S, returns the oxygen affinity of Hb S to the normal range.

Exposure of erythrocytes to high concentrations of MIC may result in methylcarbamylation of the normal Hb molecule. The resulting increased oxygen affinity of normal Hb leads to decreased oxygen dissociation, and results in relative tissue hypoxia.

In order to test these hypotheses, a series of *in vitro* experiments were undertaken. Following the *in vitro* studies, experiments using intravenous injection of liquid MIC were performed to examine whether ChE inhibition and Hb alterations were observed when MIC comes into direct contact with blood in a physiological system. Finally, *in vivo* inhalation exposures were performed to investigate the pathogenesis of mortality resulting from overexposure to MIC vapor.
Methods

In Vitro Studies

Blood samples were collected from human volunteers by venipuncture into vacutainers (Becton-Dickinson, Co., Rutherford, NJ) containing sodium heparin. Three to five milliliters of heparinized whole blood was centrifuged for 10 min at 2500 rpm and separated into plasma and packed erythrocytes. The erythrocytes were washed twice in an equal volume of physiologic saline, centrifuged for 10 min at 2500 rpm, and decanted. Approximately 1 mL of the washed erythrocytes was placed into a 30-mL Pyrex beaker containing a stirrer. The beakers were stored on ice approximately 30 min, until exposure.

Samples were exposed to statically generated MIC vapor in an inhalation chamber described by Dodd et al. (3). Control samples were exposed to air for 15 min; treated samples were exposed to target MIC concentrations of 100, 500, 1000, or 2000 ppm for 15 min. In the chamber, the beakers were placed on a Sybron Nuova 7 Stir Plate stirring at a speed setting of 2 (approximately 50 revolutions per min). Following exposure, samples were stored on ice until processed for either ChE assay or Hb electrophoresis.

Cholinesterase assays were performed on erythrocytes from heparinized blood samples using an AutoAnalyzer II C equipped with data handler (Technicon Instrument Corporation, Tarrytown, NY) using the method of Humiston and Wright (4).

Hemoglobin electrophoresis was done on citrated agar plates (Helena Laboratories, Beaumont, TX) to monitor qualitative changes in Hb molecules. Hemolysates of treated and control blood samples were prepared by adding one part of whole blood to 10 parts of hemolysate reagent containing 0.005M EDTA. Eight samples were run on each Titan® IV Citrate Agar Plate, and these were arranged so that two samples of Helena AFSC Hemo-Control were run on each plate. The use of the control material containing normal human hemoglobin (Hb A) with other human hemoglobins, fetal (Hb F), sickled (Hb S) and Hb C, provided markers for identification of treated samples with altered hemoglobin migrations. Samples were applied to the anodal end of the plate; 50V was applied to the plate for 45 min, after which the plate was stained using o-dianisidine and hydrogen peroxide (5,6).

Intravenous Injection Studies

Male Sprague-Dawley rats (Harlan, Inc., Indianapolis, IN) ranging in weight from 370 to 460 g and female Hartley guinea pigs (Hazelton Research Animals, Denver, PA) ranging in weight from 500 to 600 g were used. Rats lightly anesthetized with methoxyflurane received a bolus of liquid MIC (0.01 to 0.02 mg/kg) delivered through the tail vein. Control rats received a bolus of 0.005 mL of physiologic saline. Guinea pigs, also lightly anesthetized with methoxyflurane, were prepared for injection by exposing the internal jugular vein into which a bolus (0.01 mg/kg) of liquid MIC was delivered. Control guinea pigs were not injected. The animals were sacrificed 30 min postinjection. Methoxyflurane was used to anesthetize the animals prior to drawing 2 mL of blood from the abdominal aorta for ChE, Hb electrophoresis, or chemistry analyses. All animals were necropsied immediately following bleeding; lungs were fixed in 10% neutral buffered formalin and processed as described by Fowler et al. (7).

The Centrifichem centrifugal analyzer (Baker Instruments, Pleasantville, NY) was used to analyze plasma concentrations of creatinine kinase (CK), lactic dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and sorbitol dehydrogenase (SDH). The Astra-8 Automated Stat/Routine Analyzer (Beckman Instruments, Inc., Brea, CA) was used for analysis of plasma concentrations of sodium, potassium, and chloride.

Inhalation Studies

Male Sprague Dawley rats and male Hartley guinea pigs were exposed to MIC vapor for 15 min. Details of the chamber design, exposure conditions, and analytical procedures are described by Dodd et al. (3). The target MIC vapor concentrations were 100, 600, or 1000 ppm for rats and 25, 125, or 225 ppm for guinea pigs. Animals were sacrificed immediately following the 15 min exposure (0 hr) or at 1, 2, 4, or 16 hr postexposure. The animals were anesthetized using methoxyflurane and approximately 3 mL of blood was collected from the abdominal aorta for clinical chemistry, ion-exchange chromatography, hematology, and determination of blood gases.

Levels of the CK cardiac specific isozyme, CK-MB, were measured using a modified ion-exchange column chromatographic technique (8,9) for fraction separation followed by a quantitative assay of CK on the Centrifichem Analyzer as described above. This CK-MB analysis provides a more definitive indication of myocardial damage contributing to CK increases.

Total leukocyte count, erythrocyte count, hemoglobin, hematocrit, and erythrocyte indices, and platelets were determined with EDTA anticoagulated blood using a Coulter Counter S-Plus IV (Counter Electronics, Inc., Hialeah, FL). Wright-stained blood smears for differ-

| Table 1. Inhibition (%) of erythrocyte cholinesterase following 15 min in vitro exposure to methyl isocyanate (MIC) vapor. |
|---------------------------------------------------------------|
| Type of sample | 100 ppm | 500 ppm | 1000 ppm | 2000 ppm |
|----------------|---------|---------|----------|----------|
| Human 1        | 12a     | 27      | 68       |          |
| Human 2        | 13      | 42      | 78       |          |
| Human 3        | 4       | 34      | 61       |          |
| Human 4        | 14      | 46      | 43       |          |
| Rat*           | 10      | 0       | 37       | 88       |
| Guinea pig*    | 58      | 54      | 92       |          |

*Percent inhibition compared to pre-exposure values.  
*bNo samples at this concentration.  
*Values represent data from one animal.
Table 2. Mean hemoglobin concentration and hematocrit values in male rats following 15-min exposure to MIC vapor.

| Target MIC concentration, ppm | Time postexposure, hr | Hemoglobin (g/dL) | Hematocrit (%) |
|-------------------------------|----------------------|------------------|----------------|
|                               | 0                    |                  |                |
|                               | 13.7*                | 13.6             | 14.9           | 14.1           | 13.5           |
|                               | (0.4)                | (0.5)            | (0.3)          | (1.3)          | (0.8)          |
|                               | 100                  |                  |                |                |
|                               | 13.8                 | 13.8             | 14.4           | 14.9           | 14.3           |
|                               | (0.4)                | (0.4)            | (0.5)          | (0.3)          | (0.6)          |
|                               | 600                  |                  |                |                |
|                               | 13.9                 | 14.1             | 14.0           | 14.6           | 15.9           |
|                               | (0.8)                | (0.3)            | (0.4)          | (0.9)          | (1.2)          |
|                               | 1000                 |                  |                |                |
|                               | 13.9                 | 14.6*            | 14.8*          | 15.9*          | 16.8*          |
|                               | (0.6)                | (0.6)            | (0.4)          | (1.2)          | (1.2)          |

*Values represent mean (SD) for a group size of 7–10.

Table 4. Mean erythrocyte and mean reticulocyte percentage in male rats following 15-min exposure to MIC vapor.

| Target MIC concentration, ppm | Time postexposure, hr | Erythrocytes, 10⁶ µL | Reticulocytes, % RBC |
|-------------------------------|----------------------|----------------------|----------------------|
|                               | 0                    | 6.4                  | 6.0                  |
|                               | (0.5)                | (0.2)                | (0.3)                |
|                               | 100                  | 6.5                  | 7.1                  |
|                               | (0.4)                | (0.3)                | (0.4)                |
|                               | 600                  | 6.9                  | 6.9                  |
|                               | (0.4)                | (0.2)                | (0.5)                |
|                               | 1000                 | 6.3                  | 6.8                  |
|                               | (0.3)                | (0.3)                | (0.5)                |

*Values represent mean (SD), group size 7–10.

Table 3. Mean hemoglobin concentration and hematocrit values in male guinea pigs following 15-min exposure to MIC vapor.

| Target MIC concentration, ppm | Time postexposure, hr | Hemoglobin (g/dL) | Hematocrit (%) |
|-------------------------------|----------------------|------------------|----------------|
|                               | 0                    |                  |                |
|                               | 38.3                 | 38.2              | 40.1           | 39.4           | 37.4           |
|                               | (1.4)                | (1.5)             | (1.0)          | (4.5)          | (2.8)          |
|                               | 100                  |                  |                |                |
|                               | 38.9                 | 38.7              | 40.5           | 42.2           | 39.0           |
|                               | (1.0)                | (1.3)             | (1.9)          | (1.2)          | (2.0)          |
|                               | 600                  |                  |                |                |
|                               | 40.3                 | 39.6              | 40.1           | 42.5           | 46.5           |
|                               | (2.7)                | (1.3)             | (1.2)          | (2.4)          | (4.2)          |
|                               | 1000                 |                  |                |                |
|                               | 39.6                 | 41.2*              | 43.3*          | 46.2*          | 47.7*          |
|                               | (1.9)                | (1.7)             | (2.2)          | (5.1)          | (6.6)          |

*Values represent mean (SD), group size 7–10.

Table 5. Mean corpuscular volume values in male rats following 15-min exposure to MIC vapor.

| Target MIC concentration, ppm | Mean corpuscular volume |
|-------------------------------|------------------------|
|                               | 0                      | 59.6*                |
|                               | (3.3)                  | (1.6)                |
|                               | 100                    | 60.3                 |
|                               | (2.7)                  | (2.6)                |
|                               | 600                    | 58.2                 |
|                               | (1.7)                  | (1.2)                |
|                               | 1000                   | 62.9*                |
|                               | (1.5)                  | (2.2)                |

*Values represent mean (SD) for a group size of 7–10.

Table 2. Mean hemoglobin concentration and hematocrit values in male rats following 15-min exposure to MIC vapor.

EFFECTS OF MIC ON BLOOD CHEMISTRY AND HEMATOLOGY

entential leukocyte counts and new methylene blue-stained smears for reticulocyte counts were evaluated for all animals.

Samples for blood gases were drawn into heparinized syringes. The Corning Model 170 pH/ Blood Gas Analyzer (Corning Medical, Medfield, MA) with automatic calibrations was used to determine pH and partial pressures of carbon dioxide and oxygen.

Statistical Procedures

The results of the clinical pathology analyses were intercompared using Levene's test for homogeneity of variances (10). An analysis of variance (ANOVA) was performed on the groups with homogenous variances, and, if significant, group differences were delineated by Student's t-test. When heterogeneous variances were indicated, Welch and Brown-Forsyth ANOVAs were performed (10). If either was significant, group differ-
were inhibited by MIC in a concentration-related manner. At 1000 ppm of MIC, a 43 to 78% inhibition of human erythrocyte ChE activity was observed as compared to a pre-exposure sample. At 1000 ppm, rat ChE activity was inhibited 37%, and guinea pig ChE activity was inhibited 54%. An 88 to 92% ChE inhibition was observed in rat and guinea pig erythrocytes exposed to 2000 ppm of MIC.

Rats and guinea pigs exposed to 1000 ppm of MIC vapor by inhalation and sacrificed immediately after exposure showed no significant inhibition of erythrocyte ChE activity. When rats or guinea pigs were injected with 0.02 mg/kg or 0.01 mg/kg of liquid MIC, respectively, and sacrificed 30 min later, no ChE inhibition was observed in comparison to the individual pre-exposure values.

**Hematology**

Washed erythrocytes from humans and guinea pigs exposed to MIC vapor showed a concentration-related qualitative alteration in the electrophoretic mobility of Hb on citrated agar (Fig. 1). Hemoglobin from unexposed cells migrated as a single band, with a mobility similar to that of Hb A. A single migration band similar to that of Hb A was also observed in Hb from cells exposed to MIC concentrations below 1000 ppm. Hemoglobin from cells exposed to 1000 ppm of MIC showed two bands. The first of these bands had a mobility similar to Hb A; the second band showed a cathodic migration with mobility similar to Hb F. A single migration band with a mobility similar to Hb F was observed in Hb from cells exposed to 2000 ppm. Guinea pigs injected intravenously (jugular vein) with liquid MIC

![Table 6. Individual creatine kinase results of rats and guinea pigs injected intravenously with liquid methyl isocyanate and sacrificed 30 min postinjection.*](image)

| Species | Dose, mg/kg | Group size | Mean, IU/L (SD) | Range of values, IU/L |
|---------|-------------|------------|----------------|----------------------|
| Rat     | 0b          | 2          | 140 (18)       |                      |
|         | 0.01        | 4          | 4390 (2140–8370) |            |
|         | 0.02        | 3          | 2840 (1210–6070) |            |
| Guinea pig | 0c         | 2          | 251 (85)     |                      |
|         | 0.01        | 4          | 3130 (1368–5680) |            |
|         | 0.02        | 3          | 3788 (2017)   |                      |

*Following methoxyflurane anesthesia, rats were injected in the tail vein; guinea pigs were injected in the jugular vein.

Control guinea pigs were not injected.

Medians and quartile deviations were calculated for nonparametric data. These data were statistically analyzed by the Kruskal-Wallis test or by the Wilcoxon rank sum test as modified by Mann-Whitney (11).

**Results**

**Cholinesterase**

The ChE results from the in vitro MIC vapor exposures of packed erythrocytes are shown in Table 1. Human, rat, and guinea pig erythrocyte ChE activities were inhibited by MIC in a concentration-related manner. At 1000 ppm of MIC, a 43 to 78% inhibition of human erythrocyte ChE activity was observed as compared to a pre-exposure sample. At 1000 ppm, rat ChE activity was inhibited 37%, and guinea pig ChE activity was inhibited 54%. An 88 to 92% ChE inhibition was observed in rat and guinea pig erythrocytes exposed to 2000 ppm of MIC.

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![Figure 1. Hemoglobin electrophoresis on citrated agar plates. Helena AFSC Hemo-Control is run in two positions on each plate. (A) Three samples of human blood; pretreatment and following in vitro exposure to 2000 ppm of MIC. (B) One sample of human blood and 1 sample of guinea pig blood following in vitro exposure to 1000 and 200 ppm of MIC.](image)
FIGURE 2. Mean segmented neutrophil count in male rats following a 15-min exposure to methyl isocyanate vapor. Values represent means for a group size of 7 to 10. *p < 0.05 compared to control value.

FIGURE 3. Mean segmented neutrophil count in male guinea pigs following a 15-min exposure to methyl isocyanate vapor. Values represent means for a group size of 7 to 10. *p < 0.05 compared to control value.

FIGURE 4. Mean creatine kinase concentrations in male rats following a 15-min exposure to MIC vapor. Values represent means for a group size of 7 to 10. *p < 0.05 compared to controls of each evaluation period.
BIOLOGICAL EFFECTS OF HIGH CONCENTRATIONS OF METHYL ISOCYANATE VAPOR

Guinea Pig Creatine Phosphokinase

| HOURS | 0 ppm | 225 ppm |
|-------|-------|---------|
|       |       |         |
| 0     | 200   |         |
| 1     | 400   |         |
| 2     | 600   |         |
| 3     | 800   |         |
| 4     | 1000  |         |

FIGURE 5. Mean creatine kinase concentrations in male guinea pigs following a 15-min exposure to MIC vapor. Values represent means for a group size of 7 to 10. *p < 0.05 compared to controls at each evaluation period.

(0.01 mg/kg) and sacrificed 30 min later showed no alterations in the electrophoretic mobility of Hb. Rats and guinea pigs exposed to 1000 ppm of MIC by inhalation for 15 min and sacrificed immediately after exposure showed no alterations in the electrophoretic mobility of Hb.

In rats exposed to 1000 ppm of MIC vapor for 15 min, a 2 to 24% increase in hemoglobin concentration and a 3 to 27% increase in hematocrit were observed over the five postexposure evaluation periods (Table 2). Increases in both parameters, though of lesser magnitude, were also observed in rats exposed to 600 ppm or 100 ppm over all evaluation periods.

Guinea pigs exposed to 225 ppm showed increases of 10 to 15% in both the Hb concentration and hematocrit at 0, 1, and 2 hr postexposure; at 4 hr postexposure there was no significant change in either parameter (Table 3). In guinea pigs exposed to 125 ppm of MIC, hemoglobin concentration and hematocrit was increased approximately 25% at 16 hr (Table 3). Neither parameter showed a statistically significant change in the earlier postexposure evaluations at this concentration.

A concentration-related and time-related reticulocytosis was observed in rats exposed to high concentrations of MIC vapor (Table 4). This change occurred with a concomitant increase in mean corpuscular volume (MCV) in rats exposed to 1000 ppm of MIC, indicating the presence of a larger, immature cell population (Table 5). Guinea pigs exposed to MIC concentrations up to 225 ppm showed no increase in reticulocytes over the five postexposure evaluations.

Neutrophilia was observed in both rats and guinea pigs exposed to high concentrations of MIC vapor (1000 ppm and 225 ppm, respectively) at 1, 2, and 4 hr postexposure (Figs. 2 and 3). By 16 hr (data not shown), neutrophil counts in both species had returned to normal ranges. At 1, 2, and 4 hr postexposure, neither species exposed to low and mid-levels of MIC consistently showed any concentration or time-related alterations in segmented neutrophils. None of the other hematologic parameters evaluated showed any significant concentration or time-related alterations.

FIGURE 6. Fibrin deposition within small vessel in lung of guinea pig injected with 0.02 mg/kg MIC and sacrificed 30 min following injection. H & E, × 250.
Chemistry

Increased CK was the most significant clinical chemistry alteration observed in rats and guinea pigs injected IV with MIC. The CK values showed marked individual animal variation in rats and guinea pigs sacrificed 30 min following injection with 0.01 or 0.02 mg/kg (Table 6).

Increases in CK were also observed in both rats and guinea pigs exposed by inhalation to high concentrations of MIC vapor. When compared to a population of controls for each species, the actual percentage increase of CK in rats ranged from 30% to 300%; the increase in guinea pigs ranged from 100% to greater than 500% (Figs. 4 and 5). These increases were not entirely due to increased levels of the cardiac specific isozyme, CK-MB, since no consistent increase in this isozyme was demonstrated using the ion-exchange chromatographic technique.

Other enzymes (LDH, AST, ALT, SDH) monitored in these studies did not show consistent increases in either rats or guinea pigs exposed to the high concentrations of MIC, and therefore any observed increases were not considered treatment-related.

pH and Blood Gas

Immediately following the 15 min exposure to MIC and continuing throughout 16 hr, rats and guinea pigs exposed to high and intermediate concentrations of MIC showed a concentration-related and time-related decrease in pH and Po2 (data not shown). These alterations were further investigated by a series of controlled ventilation experiments described by Fedde et al. (12).

Gross Pathology

Both rats and guinea pigs receiving intravenous MIC liquid had froth-filled trachea when sacrificed 30 min following injection; the lesion became more severe in animals allowed to live longer. In addition, lungs were characterized by multifocal to confluent red areas. Histologically, these lungs showed deposition of fibrin within the lumina of small vessels (Fig. 6).

The gross lung lesions observed in the inhalation-exposed animals were identical to those observed in animals receiving intravenous injections. The red punctate to confluent areas were observed in both species of the high and intermediate concentration groups sacrificed at 1, 2, and 4 hr following exposure. Histologically, however, no fibrin deposition was observed. The detailed histopathology of these animals is described by Fowler et al (7).

Discussion

The relationship of ChE inhibition to the cause of death resulting from overexposure to MIC is unclear. Although in vitro experiments demonstrated a concentration-related inhibition of erythrocyte ChE activity, a similar inhibition was not observed in either animals receiving an IV dose of liquid MIC or those exposed by inhalation. Thus, the hypothesis that inhibition of ChE activity was a major contributing factor in the cause of death of MIC exposed animals was no longer considered viable.

Concentration-related qualitative changes in Hb molecules were demonstrated in erythrocytes exposed to MIC in vitro. Alterations of the electrophoretic mobility of Hb reflect qualitative changes in the Hb molecule, but need not necessarily be the result of carbamylation of hemoglobin. The electrophoretic mobility of Hb S is known to be altered following treatment with organic isocyanates such as MIC (13). MIC-treated Hb S specimens show an increase in cathodic migration, with a mobility similar to that of Hb A or Hb F. This alteration is thought to be the result of an increase in net negative charge consistent with a reaction of the organic isocyanate with the beta-chain groups.

No change in Hb electrophoretic mobility could be demonstrated in vivo in either MIC-injected or inhalation-exposed animals. Therefore, the hypothesis that a chemical alteration in the Hb molecule was an important factor causing death of animals exposed to MIC was also no longer considered viable.

A third hypothesis regarding the cause of death resulting from overexposure to MIC was that of intravascular coagulation. Increases in CK, an enzyme found predominantly in cardiac and skeletal muscles, are generally thought to be a sign of myocardial injury. The demonstration of intravascular coagulation in the injected animals suggested that the formation of microthrombi may be responsible for the gross lung lesion. The microthrombi may also have caused damage to the myocardium as reflected in the increases in CK. In order to substantiate these findings, we expected to find histologic evidence of fibrin deposition, an increase in the myocardial isozyme of CK, CK-MB, and a decrease in platelet numbers.

Although increased levels of CK were demonstrated in the inhalation MIC-exposed animals, the increase was not due to an increase in the CK-MB fraction. Thus, the cause of a significant increase in CK appears due to either generalized muscular injury associated with hypoxia or pulmonary changes associated with edema (14,15). This finding, coupled with the absence of fibrin deposition in the vessels of the lung, weakens the intravascular coagulation hypothesis. Since a concomitant decrease in platelet numbers was not observed, this hypothesis was no longer considered plausible.

The hematological alterations, i.e., increases in hemoglobin concentration and hematocrit, as well as the reticulocytosis, appear to be the result of respiratory distress. As the result of inefficient gas exchange, the animal responds by attempting to increase its oxygen carrying capacity through a mobilization of cells from tissue stores giving rise to an increase in hemoglobin and formed elements in the blood. These alterations, however, are apparently insufficient to correct the hypoxemia and associated decrease in pH. The nonspecific
increases in enzymes found in animals exposed to high concentrations of MIC appear to be related to this general hypoxemia.

Neutrophilia may be a physiological response to the stress of MIC exposure. However, the activation of the complement cascade in acute pulmonary injury is known to cause a neutrophilia after initial activation (16). Complement activation, therefore, may play a role in the pathogenesis of MIC-induced alterations (19).

The pH and blood gas changes observed in both species exposed to high concentrations of MIC indicate the occurrence of respiratory acidosis. These results have been further investigated in the studies by Fedde et al. (11) and Maginniss et al. (17). These findings also agree with those of Nemery et al. (18), who exposed rats to similar concentrations of MIC vapor in a static exposure chamber. In addition, these clinical pathology changes correlate well with the histopathologic findings in the lung described by Fowler et al. (7).

In conclusion, the hypotheses suggesting that either ChE inhibition, carbamylation of hemoglobin or intravascular coagulation are important factors in the cause of MIC-related deaths have not been substantiated in these studies. Instead, short-term exposure of rats and guinea pigs to high concentration of MIC causes morphologic changes in the lung that give rise to pH and blood gas changes which increase in severity over time.

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REFERENCES

1. Brown, W. E., Green, A. H., Karol, M. H., and Alarie, Y. C. Inhibition of cholinesterase activity by isocyanates. Toxicol. Appl. Pharmacol. 63: 45–52 (1982).
2. Lee, C. K. Methyl isocyanate as an anti-sickling agent and its reaction with hemoglobin. J. Biol. Chem. 251: 6226–6231 (1976).
3. Dodd, D. E., Frank, F. R., Fowler, E. H., Troup, C. M., and Milton, R. M. Biological effects of short-term, high-concentration exposure to methyl isocyanate. I. Study objectives and inhalation exposure design. Environ. Health Perspect. 72: 13–19 (1987).
4. Humiston, C. G., and Wright, G. J. An automated method for the determination of cholinesterase activity. Toxicol. Appl. Pharmacol. 10: 467–480 (1967).
5. Helena Laboratories, Procedure No. 17, December, 1983.
6. Schneider, R. G., and Barwick, R. Electrophoretic mobilities of mutant hemoglobins. In: Hematology. Vol. 1 (D. Seligson and R. M. Schmidt, Eds), CRC Press, Boca Raton, FL, 1979.
7. Fowler, E. H., Dodd, D. E., and Troup, C. M. Biological effects of short-term, high-concentration exposure to methyl isocyanate. V. Morphologic evaluation of rat and guinea pig lungs. Environ. Health Perspect. 72: 39–44 (1987).
8. Baker Instruments, Procedure RD595, August, 1983.
9. Rosenberg, B. J., Mihalik, M. C., and Gross, M. S. A rapid batch procedure for quantitation of creatine kinase. Clin. Chem. 24: 988 (1978).
10. Dixon, W. J. BMDP Statistical Software. University of California Press, Berkeley, CA, 1981.
11. Sokal, R. R., and Rohlf, F. J. Biometry. W. H. Freeman and Company, San Francisco, CA, 1981.
12. Fedde, M. R., Dodd, D. E., Troup, C. M., and Fowler, E. H. Biological effects of short-term, high-concentration exposure to methyl isocyanate. III. Influence on gas exchange in the guinea pig lung. Environ. Health Perspect. 72: 29–33 (1987).
13. Wilson, E., and Winter, W. F. Use of citrate agar electrophoresis in evaluation of antisickling agents. J. Natl. Med. Assoc. 73: 111–116 (1981).
14. Tietz, N. W. Fundamentals of Clinical Chemistry. W. B. Saunders Co., Philadelphia, PA, 1970.
15. Henry, P. D., Bloor, C. M., and Sobel, B. E. Increased serum creatine phosphokinase activity in experimental pulmonary embolism. Am. J. Cardiol. 26: 151–155 (1970).
16. Till, G. O., and Ward, P. A. Systemic complement activation and acute lung injury. Fed. Proc. 45: 13–18 (1986).
17. Maginniss, L. A., Szewczak, J. M., and Troup, C. M. Biological effects of short-term, high-concentration exposure to methyl isocyanate. IV. Influence on oxygen binding properties of guinea pig blood. Environ. Health Perspect. 72: 35–38 (1987).
18. Nemery, B., Dinsdale, D., Sparrow, S., and Ray, D. E. Effects of methyl isocyanate on the respiratory tract of rats. Br. J. Ind. Med. 42: 799–805 (1985).
19. Kolb, W. P., Savary, J. R., Troup, C. M., Dodd, D. E., and Tamerius, J. D. Biological effects of short-term, high-concentration exposure to methyl isocyanate. VI. In vitro and in vivo complement activation studies. Environ. Health Perspect. 72: 189–195 (1987).