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Biological Effects of Short-Term, High-Concentration Exposure to Methyl Isocyanate. I. Study Objectives and Inhalation Exposure Design

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Early reports from India indicated that humans were dying within minutes to a few hours from exposure to methyl isocyanate (MIC). Attempts to explain the cause(s) of these rapid mortalities is where Union Carbide Corporation concentrated its post-Bhopal toxicologic investigations. The MIC studies involving rats and guinea pigs focused primarily on the consequences of acute pulmonary damage. All MIC inhalation exposures were acute, of short duration (mainly 15 min), and high in concentration (ranging from 25–3506 ppm). MIC vapors were statically generated in a double chamber exposure design. Precautionary measures taken during exposures are discussed. Guinea pigs were more susceptible than rats to MIC exposure-related early mortality. A greater than one order of magnitude difference was observed between an MIC concentration that caused no early mortality in rats (3506 ppm) and an MIC concentration that caused partial (6%) early mortality in guinea pigs (225 ppm) for exposures of 10 to 15 min duration. For both species, the most noteworthy clinical signs during exposure were lacrimation, blepharospasm, and mouth breathing. Fifteen minute LC_{50} tests with 14-day postexposure follow-up were conducted, and the LC_{50} (95% confidence limit) values were 171 (114–256) ppm for rats and 112 (61–204) ppm for guinea pigs. Target exposure concentrations for the toxicologic investigations of MIC-induced early mortality were established. A short summary of pertinent results of Union Carbide Corporation's post-Bhopal toxicologic investigations is presented.

Introduction

Background

Union Carbide Corporation first tested methyl isocyanate (MIC) for acute and other types of toxic effects, including those found with low dose human exposure, in the early 1960s (*1–3*). MIC caused severe necrosis of the skin and eyes, had a low peroral LD_{50}, and was a potent sensitizing agent in the guinea pig. Human exposure to 1.75 ppm MIC for 1 min resulted in eye irritation and lacrimation in all those exposed, and nose and throat irritation in approximately one-half of the eight subjects. All effects disappeared within 10 min following termination of the exposure.

Due to the limited design of these early studies, the less than precise analytical techniques available in the 1960s, and the fact that MIC had been used to produce commercial carbamylated pesticides, further acute testing in several species and repeated dose testing in rats, all by inhalation exposure, was done by Union Carbide Corporation in the early 1980s (*4–6*). A discussion of the results of these MIC studies have been included in this issue of *Environmental Health Perspectives* (*7–9*). There was good agreement between the results of the studies performed in the 1960s and the studies conducted in the 1980s (*4*).

Current Study Objectives and Specific Areas of Concern

Since the Bhopal incident, Union Carbide Corporation has performed numerous toxicologic investigations with MIC. The overall objective of these studies was to determine the consequences of pulmonary injury following an acute exposure of high MIC vapor concentration and to elucidate the pathogenesis of early mortality. These studies were intended to complement, but not duplicate, the National Institute of Environmental Health Sciences MIC studies initiated in 1985. Numerous early reports from the media indicated that humans were dying within minutes to a few hours from exposure to the material that drifted over Bhopal. There were a number of symptoms described and opinions rendered

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as to why these deaths occurred. An attempt to explain the cause of these early mortalities is where Union Carbide Corporation concentrated its efforts.

Since previous studies (*4,5*) indicated the guinea pig to be the most sensitive species to acute MIC exposure as compared to rats and mice, the guinea pig was selected for the present investigations to focus on the pathogenesis of early mortality. The rat was also selected to ascertain any species differences related to MIC exposure. Early mortality was defined as death occurring within 4 hr following a single MIC exposure of 15 min duration.

Many of the symptoms of Bhopal victims could have fit any or all of several pathophysiologic disturbances which, if severe enough, could result in rapid death. Union Carbide Corporation chose to investigate the following pathophysiologic mechanisms: (1) an inhibition of cholinesterase activity, (2) an alteration of hemoglobin function, (3) gas exchange impairment in the lung, (4) morphologic alteration of the lung compromising gas exchange, (5) development of disseminated intravascular coagulation, and (6) activation of the complement system resulting in adult respiratory distress syndrome.

Methyl isocyanate is used as a chemical intermediate in the production of carbamate pesticides, several of which are potent, though reversible, cholinesterase inhibitors (*10*). Brown et al. (*11*) have observed the inhibition of cholinesterase activity by several diisocyanates and isocyanates. Thus, erythrocyte cholinesterase activity was measured in the present studies following both *in vitro* and *in vivo* MIC exposure (*12*).

Hemoglobin alteration by MIC was also studied (*12,13*) because isocyanates, including MIC, and cyanates are known to increase the oxygen affinity of the hemoglobin of persons with sickle cell anemia, returning their hemoglobin oxygen affinity to the range of normal hemoglobin (*14–17*). It was postulated that carbamylation of normal hemoglobin could increase oxygen affinity to a point where the oxygen would not be released in peripheral circulation and result in hypoxia at the tissue level. In previous studies (*6*), a decrease in oxygen content of hemoglobin and an increase in hemoglobin concentration were observed in rats repeatedly exposed to 3.1 ppm of MIC.

During the course of these investigations on hemoglobin, a variety of respiratory parameters, including blood gas partial pressures, pH, oxygen saturation, oxygen content, as well as serum chemistry and hematologic parameters, were measured (*12*). The results indicated the development of a condition of severe acidosis. This led to a series of controlled ventilation experiments with guinea pigs to further study the effect of MIC on the gas exchange functions of the lungs (*18*). Also, the effect of exposure on the oxyhemoglobin dissociation curve was explored to assist in interpreting the respiratory function data (*13*).

A few experiments with MIC were performed by administering liquid MIC intravascularly (*12*). The results of this work suggested the occurrence of intravascular coagulation. This finding, coupled with the observation of increased blood creatine phosphokinase levels, suggested that MIC caused a condition of localized intravascular coagulation resulting in myocardial ischemia and mortality. This hypothesis was tested in the rat (*12*).

Recent medical research is associating activation of the human complement system with the condition known as adult respiratory distress syndrome (*19–21*). In systemic complement activation, the C3A and C5A protein fragments that are released when either the classic or alternate complement pathways are activated are potent anaphylatoxins. C5A is particularly potent and among other actions, releases histamines from cells. The release of C5A anaphylatoxin can be quickly lethal under certain conditions. MIC activation of the complement system was investigated to determine what role it may have in MIC toxicity (*22*).

Finally, the morphology of the respiratory system, with emphasis on the gas exchange regions of the lungs, was examined in rats and guinea pigs acutely exposed to high concentrations of MIC vapor (*23*). Wherever possible, a correlation between lung morphology and hematologic, blood gas, serum chemistry, and complement-related changes was provided.

Methods

Test Material

Liquid MIC (CAS No. 624-83-9) was obtained from either Union Carbide Corporation, Institute Plant (South Charleston, WV) or Aldrich Chemical Company, Inc. (Milwaukee, WI).

Inhalation Chambers and MIC Vapor Generation

Animals were placed into a suspended, stainless-steel, wire-mesh cage (approximately 450 cm^2 floor space), which was part of a sliding drawer mechanism on a rectangular-shaped 135-L Plexiglas chamber (Fig. 1). A stainless-steel tray was placed on the chamber floor and a mixing fan was attached to one wall of the chamber. For *in vitro* vapor exposures of biological specimens, the samples were placed on a magnetic stirrer which was positioned on the chamber floor. All MIC vapor exposures were statically generated (i.e., air was not passed through the chamber during exposure). The test material was introduced into the chamber with a glass syringe through a 1/4 inch stainless-steel bulkhead sampling port containing a gas chromatograph septum. Following injection of liquid MIC into the chamber, evaporation of the sample occurred in a matter of seconds. Chamber concentration reached equilibrium within approximately 1 min. The sliding cage drawer mechanism prevented the animals' introduction into the chamber until mixing of the MIC with air had equilibrated. Thus, chamber concentrations were determined prior to animal exposure. For vapor exposures of the

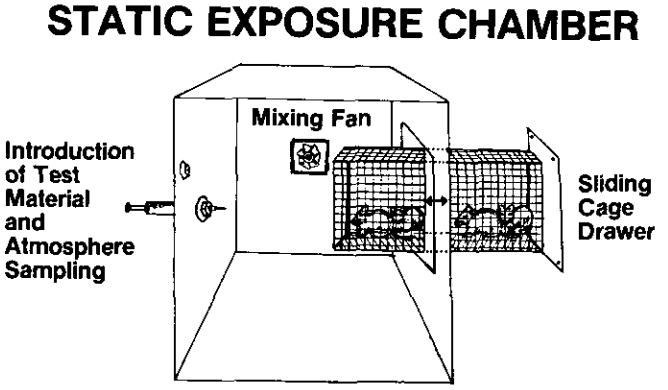

FIGURE 1. A static exposure chamber with sliding cage drawer mechanism was used to expose rats and guinea pigs to atmospheres containing methyl isocyanate vapor.

biological specimens, the samples were placed into the chamber prior to the introduction of MIC, and therefore, chamber equilibrium time was part of the exposure time. Chamber temperature and relative humidity were also monitored during exposures.

Analytical Determination of Chamber Concentration of MIC Vapor

Chamber air was sampled manually with a glass gas-tight syringe two to four times per exposure. The 1-mL air samples were injected into a Perkin-Elmer 3920B gas chromatograph (GC) equipped with a flame ionization detector. Attached to the GC was a Spectra Physics Series 4000 central processor, data interface, and a printer/plotter. A 3-ft × 1/4 in. stainless-steel column packed with Chromosorb 101 (80/100 mesh) support was used. Calibration of the GC was done with gas bag standards which were prepared by injecting a known quantity of liquid MIC into a Tedlar gas sample bag of known volume. A linear calibration curve was obtained when areas (integration counts) were plotted versus the concentrations of the standards. The approximate minimum detection limit was 1 ppm of MIC.

The analytical to nominal (A/N) chamber concentrations of MIC for the static exposures ranged between 0.5 and 0.9. The nominal concentration was determined by dividing the amount of liquid MIC placed into the chamber by the chamber volume. One explanation for the low A/N ratios was the loss of MIC vapor when animals were introduced into the chamber via the sliding cage drawer mechanism (Fig. 1). The high reactivity of MIC may also explain some chamber losses of concentration. In addition, chamber animal load correlated well with the A/N ratio. For example, experiments involving one to two animals per exposure had A/N ratios of approximately 0.75, while those involving four to five animals per exposure had A/N ratios of approximately 0.6. The rate of decay of MIC concentration during exposure was, in general, not greater than 10% during the 15-min exposures. The factor which appeared most closely associated with the rate of decay was the amount of animal urination. Thus, the rate of decay of MIC chamber concentration was greater during guinea pig exposures than rat exposures, since a higher incidence of urination, as well as a greater amount of urination, occurred in the guinea pigs.

Precautionary Measures During MIC Exposure

MIC is a flammable, reactive, volatile, and highly toxic chemical; therefore, numerous precautionary measures were taken prior to the initiation of exposures and during MIC exposures. The exposure chamber illustrated in Fig. 1 was placed in a dynamic 900-L exposure chamber, constructed of stainless-steel and glass, providing a double chamber exposure design (Fig. 2). The dynamic exposure chamber was operated at an airflow of approximately 350 L/min. This chamber contains two glove ports on each of two opposing walls which allows two people to simultaneously perform operations inside the chamber. The injection of liquid MIC into the static exposure chamber, the introduction and removal of animals through the sliding drawer mechanism, and the decontamination of MIC vapor within the static exposure chamber were performed by workers standing outside of the dynamic exposure chamber.

Attached to the dynamic exposure chamber was a 940-L stainless-steel and glass glove-box operated with a dynamic airflow of approximately 350 L/min (Fig. 2). A hinged air-tight door separates the dynamic exposure chamber from the glove box. The glove box is equipped with four glove ports on each of two opposing walls, and on the far end of the glove box is another hinged air-tight door, which opens into the room containing the exposure chamber/glove box assembly. Both the chamber and the glove box are maintained at a negative

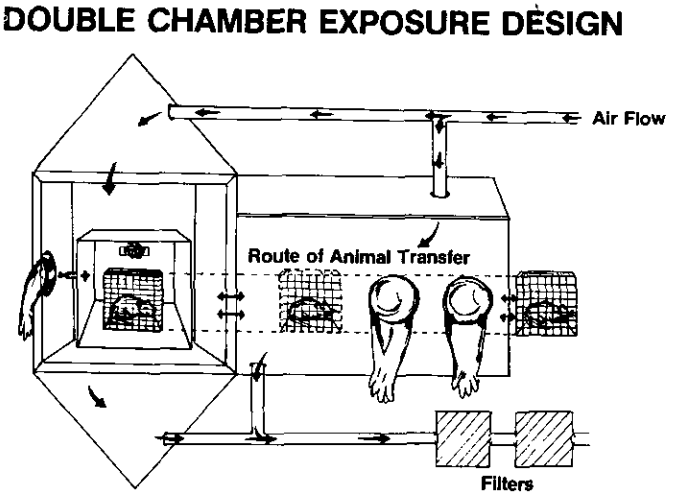

FIGURE 2. A static exposure chamber was placed in a dynamic exposure chamber/glove box assembly providing a double chamber exposure design to expose rats and guinea pigs to atmospheres containing methyl isocyanate vapor.

pressure with respect to the room. This inhalation exposure system (Figs. 1 and 2) provided the means for quick and easy removal of MIC-exposed animals without contaminating the personnel involved in vapor exposure operations. Within 1 min following exposure, animals could be removed from the double chamber system without release of MIC vapor into the workplace.

The static MIC exposure chamber was decontaminated by diluting the vapor with air passing through the dynamic exposure chamber. The exhaust from the dynamic exposure chamber/glove box assembly was filtered with two types of activated carbon, whetlerized CG and standard type VG (Barnabey-Cheney, Columbus, OH), prior to release from the exhaust stack. During exposures, the exhaust stack was monitored for MIC vapor with a Perkin-Elmer gas sampling system (described below). No MIC was detected at the exhaust stack, indicating the filtration system was adequate for scrubbing MIC vapor.

Additional precautionary measures included a high speed exhaust fan attached to the room containing the exposure chamber/glove box assembly which provided the workplace with a high number of air changes and kept the room under negative pressure compared to the rest of the laboratory. Positive-pressure, full-face air respirators were available in case of emergencies, as well as backup chamber exhaust fans and emergency power generation in case of laboratory power failure. During inhalation exposures, sites surrounding the exposure chamber area were monitored for MIC. Air sampling was performed automatically with a Perkin-Elmer gas sampling system (station and valve programmer units and a gas sampling valve) attached to a Perkin-Elmer 3920B GC equipped with a nitrogen-phosphorus detector. A Chromosorb 101 stainless-steel column (4 ft × 1/4 in.) was used for the analysis. A real-time analysis of approximately 30 parts per billion (pbb) was achieved with this system. The GC was calibrated using MIC permeation tubes and a VICI Metronics Dynacalibrator 450. The permeation rate of the permeation tubes was determined gravimetrically.

Animals and Exposure Conditions

Specific pathogen-free (SPF) Sprague-Dawley rats (200–300 g for males and 195–265 g for females) were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and SPF Hartley strain guinea pigs (300–650 g for the majority of experiments) were obtained from Hazleton Research Animals (Denver, PA). All MIC inhalation exposure conditions were acute, of short duration (mainly 15 min) and high in concentration (a target concentration range of 25–3500 ppm). In general, rats were exposed in groups of four to five and guinea pigs in groups of three to four, although for some experiments guinea pigs were exposed individually or in pairs (*13,18,22*). Food and water were provided *ad libitum* except during inhalation exposures. In several experiments, control animals or control biological specimens were exposed statically to air alone to simulate the MIC exposure conditions. However, in other experiments, control animals were not exposed in chambers to air alone, but simply removed from their housing quarters. Standard husbandry conditions were maintained.

Results and Discussion

LC_{50} Determinations

Fifteen-minute LC_{50} values with the traditional 14-day postexposure observation period were determined for female rats and female guinea pigs. The LC_{50} (95% confidence limit) values were 171 (114–256) ppm and 112 (61–204) ppm for rats and guinea pigs, respectively. These values are consistent with data from past studies (*3,4,24*). The 15-min LC_{50} values are also in agreement with the observation that the guinea pig is more sensitive than the rat following MIC exposure (*4,5,7*).

Early Mortality Observations

The approximate time of death for rats and guinea pigs in the 15-min LC_{50} study was 1 to 3 days postexposure. To define the appropriate exposure conditions for elucidating the mechanism(s) of early mortality, the next objective was to examine what MIC concentration was necessary to cause mortality during or soon after exposure. The early mortality results of rats and guinea pigs exposed to MIC concentrations ranging from 225 to 3506 ppm for exposure periods of 10 to 20 min are presented in Table 1. Guinea pigs were clearly more susceptible than rats to MIC exposure-induced early mortality.

No apparent mortality differences between sexes were observed for either rats or guinea pigs (data not shown). No rats died during or within 10 min postexposure for MIC exposure conditions as high as 3506 ppm and 10 min in duration. However, complete group mor-

Table 1. Early mortality of rats and guinea pigs exposed to methyl isocyanate vapor.

MIC concentration, ppm	Exposure time, min	Deaths during exposure or within 10 min postexposure	
		Rats	Guinea pigs
3506	10	0/5 (0%)[a]	
2099	10	0/5 (0%)	
1930	20	0/5 (0%)	
1516	15		4/4 (100%)
1100	10	0/4 (0%)	
1000[b]	15	0/166 (0%)	6/9 (67%)
821	15		3/4 (75%)
675[b]	11–15		17/40 (43%)
600[b]	15	0/120 (0%)	
530	15		6/8 (75%)
350	15		1/8 (13%)
225[b]	15		4/65 (6%)

[a] Each ratio reflects the number of deaths/the number exposed. Numbers in parentheses indicate mortality percentage.

[b] These are target MIC concentrations. The actual exposure concentrations were ± 10% of the target concentration.

tality was observed in guinea pigs exposed to 1516 ppm of MIC for 15 min. Thus, a greater than one order of magnitude difference exists between an MIC concentration that caused no early mortality in rats (3506 ppm) and an MIC concentration that caused partial (6%) early mortality in guinea pigs (225 ppm) for exposures of 10 to 15 min duration. Nemery et al. (*25*) observed early mortality in LAC-P rats exposed for 15 min to an MIC concentration of 10 mg/L (approximately 4300 ppm). Their findings, as well as the results of the current study, indicate early mortality occurs in rats at MIC concentrations considerably higher than those causing early deaths in guinea pigs.

Noteworthy clinical signs observed in rats and guinea pigs exposed to these high (≥225 ppm) MIC concentrations were lacrimation, nasal wetness, rubbing of eyes and nose with forepaws, partial to complete closure of eyelids, salivation, and mouth breathing. A decrease in respiratory rate (qualitatively assessed) was common for both species. These clinical signs appeared quickly (1–3 min), and mouth breathing persisted for several hours following exposure. Guinea pigs appeared more restless than rats, and short periods (5–15 sec) of hyperactivity were observed in the guinea pigs, although convulsions were not observed. Prior to death, animals were prostrate and gasping. The time between gasps varied, but in general, increased as the moment of death approached. Animals exposed to MIC did not appear unconscious, but rats appeared hypoactive. Salmon et al. (*26*) observed a pronounced narcotic or sedative effect in male Lister hooded rats exposed to low concentrations of MIC (e.g., 11 ppm); however, this effect was not observed at higher MIC concentrations, presumably due to the arousal resulting from severe irritation and respiratory distress.

To elucidate the cause(s) of early mortality, MIC exposure conditions were selected that would allow a majority of a group of exposed animals to survive a few hours postexposure so that blood and tissue samples could be obtained for toxicologic evaluations. As mentioned previously, early mortality was defined as death occurring within 4 hr following a single MIC exposure of 15 min duration. Table 2 presents the time of death and the percentage of mortality for guinea pigs exposed to target MIC concentrations ranging from 25 to 225 ppm and for rats exposed from 100 to 1000 ppm. All exposures were 15 min in duration. The highest MIC concentrations (1000 ppm for rats and 225 ppm for guinea pigs) caused 69 to 100% mortality between 4 and 16 hr postexposure. An accurate determination of the percentage of mortality between 0 hr (immediately following exposure) and 4 hr postexposure could not be made because animals were sacrificed at specified intervals postexposure (0, 1, 2, and 4 hr). The lowest MIC concentrations (100 ppm for rats and 25 ppm for guinea pigs) caused no mortality between 4 and 16 hr postexposure (Table 2) and were considered unlikely to cause any deaths since these concentrations were below the lower limit of the 95% confidence interval of the 15-min LC_{50} values. MIC concentrations that caused approximately 50% mortality 4 to 16 hr postexposure were selected for the intermediate exposure concentrations. The rat to guinea pig concentration ratio for the three target MIC concentrations ranged from 4.0 to 5.0.

Table 2. Percentage of mortality and time of death for rats and guinea pigs exposed to high methyl isocyanate concentrations for 15-min periods.

Species	MIC concentration, ppm[a]	% Mortality, Time postexposure: 10 min[b]	% Mortality, Time postexposure: 4–16 hr[c]
Rat	1000	0	69
	600	0	47
	100	0	0
Guinea pig	225	6	100
	125	0	58
	25	0	0

[a] Target MIC concentrations. Actual concentrations were ± 10% of target concentrations, 15-min exposure.
[b] Includes mortalities occurring during exposure.
[c] Mortality = number of animals found dead 4–16 hr postexposure/number of survivors at 4 hr postexposure × 100. Animals found dead or sacrificed before 4 hr postexposure were not included in this calculation.

Several studies performed by Union Carbide Corporation (*13,18,22*) involved the exposure of guinea pigs to a target MIC concentration of 675 ppm for 15 min. This exposure condition caused approximately 50% mortality during or within 10 min postexposure (Table 1). The purpose of these investigations was to maximize the opportunity for MIC to gain entry into the animal's vascular system via inhalation and to determine any alterations in specific proteins, such as hemoglobin or complement, which may have contributed to the cause of sudden death.

Summary of Union Carbide Corporation's Post-Bhopal Toxicologic Investigations

Early reports from Bhopal indicated that humans were dying within minutes to a few hours from exposure to MIC. To examine the probable causes of these rapid mortalities, studies involving rats and guinea pigs focused primarily on the consequences of acute pulmonary damage. All MIC inhalation exposures were acute, of short duration (mainly 15 min) and high in concentration (ranging from 25 to 3506 ppm). The MIC vapor exposures were statically generated in a double chamber design. Guinea pigs were more susceptible than rats to exposure-related early mortality. A greater than one order of magnitude difference was observed between an MIC concentration that caused no early mortality in rats (3506 ppm) and an MIC concentration that caused partial (6%) early mortality in guinea pigs (225 ppm). Early mortality was defined as death occurring within 4 hr following a single MIC exposure of 15 min duration.

Although human, rat, and guinea pig packed erythrocytes exposed *in vitro* to 100, 500, 1000, or 2000 ppm of MIC vapor had a concentration-related inhibition of

cholinesterase activity, *in vivo* exposure of rats and guinea pigs to 1000 ppm of MIC did not result in inhibition of erythrocyte cholinesterase (*12*). Additional noteworthy alterations in blood of rats and guinea pigs exposed *in vivo* to high concentrations of MIC vapor were an increase in creatine kinase, increases in hemoglobin concentration and hematocrit, reticulocytosis (rats only), and neutrophilia. No direct effects of MIC on hemoglobin function were observed in guinea pigs exposed to 700 ppm for 15 min (*13*). However, blood O_2 affinity was reduced due to severe metabolic acid-base disturbances (lactic acidosis).

Guinea pigs exposed to MIC at concentrations of 240 to 628 ppm had a marked reduction in PaO_2 and pHa and an elevated tracheal pressure during artificial ventilation (*18*). The low PaO_2 was only slightly elevated when the animals were ventilated with 100% O_2. Thus, MIC inhalation caused severe pulmonary blood shunting and ventilation/perfusion imbalance. This, in turn, led to hypoxemia, metabolic acidosis, and tissue hypoxia, which could produce death. The pulmonary gas exchange deficit presumably resulted from sloughing of large sheets of conducting airway epithelium together with fibrin buildup and increased mucus production, resulting in plugging of major airways and atelectasis (*23*). The severity of morphological changes was correlated with exposure concentration and time postexposure in both rats and guinea pigs. Degenerative changes were observed in the bronchial, bronchiolar, and alveolar epithelium, as well as the endothelium in both species. However, the guinea pig was considerably more sensitive to MIC than was the rat.

Results of experiments in which animals received intravenous doses of liquid MIC suggested that disseminated intravascular coagulation may be responsible for MIC-induced early mortality (*12,23*). However, in MIC vapor-exposed animals, the evidence was not strong enough to support this hypothesis.

The *in vitro* exposure of human or guinea pig serum to MIC vapor induced profound alterations in the complement system (*22*). These complement alterations resulted in reduction of several complement component functional activities, with the guinea pig complement system being more sensitive to inactivation than the human. Results were also obtained which indicated that complement activation occurred *in vivo* when guinea pigs were exposed to MIC vapor for short time periods. The guinea pig complement consumption profile observed *in vivo* was qualitatively similar to that seen *in vitro*.

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