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Respiratory Tract Changes in Guinea Pigs, Rats, and Mice Following a Single Six-Hour Exposure to Methyl Isocyanate Vapor

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Groups of male and female Fischer 344 rats, B6C3F1 mice, and Hartley guinea pigs were exposed once for 6 hr to mean concentrations of 10.5, 5.4, 2.4, 1.0, or 0 (control) ppm of methyl isocyanate (MIC) vapor. Rats and mice were also exposed to 20.4 ppm of MIC. The majority of deaths occurred during postexposure days 1 through 3. The 6-hr LC_{50} values (with 95% confidence limits) were 6.1 (4.6 to 8.2) ppm for rats, 12.2 (8.4 to 17.5) ppm for mice, and 5.4 (4.4 to 6.7) ppm for guinea pigs. Notable clinical observations during and immediately following MIC exposure were lacrimation, perinasal/perioral wetness, respiratory difficulty (e.g., mouth breathing), decreased activity, ataxia, and hypothermia. Body weight losses were common in all species following MIC exposures of 2.4 ppm or greater. Microscopic lesions included acute necrosis of the epithelial lining throughout the respiratory tract in animals that died shortly after exposure, coupled with congestion, edema, and inflammation. A microscopic lesion that appeared unique to guinea pigs was bronchiolitis obliterans (where the products of necrosis and inflammation completely closed the bronchioles). Additional microscopic lesions observed in some animals that died or were sacrificed at the end of the study (postexposure day 14) consisted of squamous metaplasia of respiratory epithelium in the nasal cavity, which extended into the larynx, trachea, and in some cases, the bronchi. In addition, epithelial regeneration throughout the tract and submucosal fibroplasia in the trachea, bronchi, and bronchioles were observed, the latter lesion being primarily confined to rodents. Only in guinea pigs were there lesions in the 1.0 ppm group attributed to MIC exposure. In conclusion, guinea pigs were more sensitive to the MIC vapor than were rats, which were in turn more sensitive than mice.

Introduction

The objectives of the present MIC inhalation studies, conducted in the early 1980s prior to the Bhopal tragedy, were: (1) to update the acute toxicity profile using state-of-the-art analytical equipment and procedures, (2) to expand the acute toxicity profile by determining LC_{50} values in three species, (3) to gain insight into the type and severity of lesions occurring in the respiratory tract of three species as a result of a single exposure, and (4) to predict target exposure concentrations for future acute or repeated exposure inhalation studies.

Hartley guinea pigs, Fischer 344 rats, and B6C3F1 mice of both sexes were exposed once to concentrations of 10.5, 5.4, 2.4, 1.0, or 0.0 (control) ppm of methyl isocyanate (MIC) vapor in order to determine the 6-hr LC_{50}. Additionally, the rats and mice were exposed to an MIC vapor concentration of 20.4 ppm. A previous 4-hr LC_{50} study involving guinea pigs had indicated that this species could not survive the higher concentration (*1*). Few other reported studies have examined the acute toxicity of MIC in laboratory rodents (*2–4*).

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Materials and Methods

Details of the exposure methodology, vapor generation methods, and analytical procedures used in this study have been described previously (*1*). Briefly, vapors of MIC were generated by passing nitrogen gas through a stainless-steel cylinder containing liquid MIC. The MIC vapors were metered into stainless-steel and glass chambers (4350 L) operated at an airflow of 1000 L/min. Chamber air was analyzed for MIC two to five times per hour with a Perkin-Elmer 3920B gas chromatograph equipped with a nitrogen/phosphorus detector. A Spectra-Physics series 4000 central processor, a data interface, and a printer-plotter, in addition to a Perkin-Elmer automatic gas sampling system, were used for the analyses. Calibration of the gas chromatograph was performed with liquid standards of MIC in *n*-hexane. The minimum detection limit was approximately 100 ppb.

Animal Species, Source, Quality Control, and Animal Husbandry

Fischer 344 rats [COBS® CDF®(F344)/CrlBR] and B6C3F1 mice [COBS® B6C3F1 /CrlBR], 32 to 34 days

Table 1. Chamber analytical concentrations of methyl isocyanate for the acute 6-hr exposures.

Mean concentration, ppm	Standard deviation	Concentration range during exposure, ppm
20.4	0.6	19.5–22.4
10.5	0.3	9.8–11.1
5.4	0.5	4.8–6.8
2.4	0.3	1.6–2.8
1.0	0.1	0.7–1.1
0.0	0.0	0.0–0.0

old, were received from Charles River Breeding Laboratory (Portage, MI). Hartley guinea pigs, 4 weeks old, were received from Hilltop Laboratory Inc. (Scottdale, PA). Upon arrival, fecal samples were examined for intestinal parasites by zinc sulfate flotation. The results of the quality control examinations were negative. All animals were examined prior to initiation of exposure to ensure that the animals were in good health. Body weight determinations were also performed prior to the initiation of exposures. Rats and mice were housed three per cage in stainless-steel, wire-mesh cages. Guinea pigs were housed two per cage in similar cages. A layer of Deotized Animal Cage Board® (Shepherd Specialty Papers, Inc., Kalamazoo, MI) was placed under each row of cages. Temperature and relative humidity were recorded continuously (Cole-Parmer Hygrothermograph Seven-Day Continuous Recorder, Model #8368-00). Animals were kept on a 12-hr photoperiod throughout the study. Water was supplied to rats by an automatic watering system, to mice by both water bottles and automatic watering system, and to guinea pigs by water bottles only. A certified powdered rodent feed (Purina #5002) was supplied by Ralston Purina Company (Richmond, IN). Guinea pig feed was supplied by Country Foods—Division of Agway Inc. (St. Marys, OH). Food and water were available to animals *ad libitum*. During the inhalation exposure, animals were grouped three per cage (separated by species, sex, and test group) in wire-mesh, stainless-steel cages. Food and water were withheld and cageboard was not placed under the cages during the exposures.

Animal Identification and Group Assignment

Each animal was distinctly numbered by toe clipping or ear tagging (guinea pigs). Body weights and physical condition of all animals were followed for approximately 5 weeks prior to placement into exposure groups. Animals were assigned to five test groups (six/sex/species) by employing a card-based random number system. At the time of group assignment, only animals with body weights within two standard deviations of the group mean for each sex and species were used in the study. Any animal in poor health was rejected from group assignment.

Target Concentrations and Exposure Regimen

Target concentrations of 20, 10, 5, 2.5, 1.0, and 0 ppm were selected for this study. All exposures were for 6 hr. Control (air-exposed) animals were handled in an identical manner as the MIC-treated animals. All survivors were maintained for a 14 day observation period following exposure.

Biological Evaluations

Animal Observations and Body Weights

All animals were observed following each exposure and once every postexposure day for signs of toxicity. Abnormal apearances were recorded at these times. Animals were weighed on the morning preceding MIC exposure, on the mornings of the first and seventh days after exposure and again preceding sacrifice.

Necropsy and Pathology

Animals that died following the 6-hr exposure were necropsied as found. All survivors were sacrificed and necropsied 2 weeks following exposure. The animals were anesthetized with methoxyflurane and killed by severing the brachial vessels to permit exsanguination.

All animals underwent a complete necropsy with the following tissues saved in 10% neutral buffered formalin (NBF) for histopathologic examination: nasal tissues, larynx, trachea, lungs, and gross lesions. Following removal, the lungs were gently infused with 10% NBF administered intratracheally.

Following decalcification of the head, the nasal tissues were processed for examination by cutting the head at four separate levels and processing four blocks of tissue (*5*). Other tissues were trimmed, embedded in parafin; sectioned at 5 μm, and stained with hematoxylin and eosin for examination.

Statistical Analysis

Body weight results among the MIC concentration groups were compared with those of the control group by use of Bartlett's homogeneity of variance (*6*), analysis of variance (ANOVA), and Duncan's multiple range test (*7–9*). The multiple range test was used when a significant F value from an ANOVA was observed. For heterogeneous group variances, the F-test (*6*) and either Student's t-test or Cochran t-test (*10*) were used. The fiducial limit of 0.05 (two-tail) was used as the critical level of significance. The LC_{50} value with 95% confidence limits was determined by a modified method of Finney's (*11*) probit analysis. The mortality of males and females was combined in order to calculate the LC_{50} value for each species.

Results and Discussion

Chamber Concentrations and Environmental Conditions

The chamber analytical concentrations of MIC are presented in Table 1. Nominal concentrations were not calculated because the concentration of the MIC in the head space of the generation cylinder was not determined. No overlapping of MIC concentrations occurred between exposure levels. Chamber temperature and relative humidity for all exposure groups ranged from 72 to 79°F and 33 to 54%, respectively. The housing quarters for the animals were maintained within a temperature range of 65 to 78°F and a relative humidity range of 25 to 45% throughout the 14-day postexposure observation period.

Animal Observations During Exposure

Lacrimation was the most common observation and was found in at least one species during each MIC exposure. Mouth breathing was noticed in both sexes exposed to 20.4 (rats and mice only), 10.5, (rats, mice, and guinea pigs), or 5.4 ppm (mice only). Nasal discharge or wetness was seen in the 10.5 and 5.4 ppm-exposed guinea pigs and in rats of the 20.4 ppm group. Mice of the 20.4 ppm group had perioral wetness. These clinical signs of irritancy were similar to the responses reported by human volunteers exposed to MIC (*12*, *13*).

Animal Observations 30 to 60 Minutes Following Exposure

In general, both sexes were equally affected, and clinical abnormalities were more common in rats from the 20.4 and 10.5 ppm groups than rats from lower MIC concentration groups. Clinical signs included wetness around the eyes, nose, and mouth, as well as ataxia and decreased activity. Rats of the 1.0 ppm and air-control groups appeared normal. Male and female mice exhibited similar clinical signs to those of rats, but also displayed breathing difficulties and were hypothermic. Abnormal observations were more common in the 20.4 and 10.5 ppm groups compared to lower MIC concentration groups. One male mouse of the 20.4 ppm group had a convulsion. Mice of the 2.4, 1.0, and 0 ppm exposure levels appeared normal.

Adverse clinical signs, approximately 30 to 60 min following exposure, were minimal for guinea pigs. Wetness around the eyes or nose was observed in a few guinea pigs (10.5 and 5.4 ppm exposure groups). The remaining MIC-exposed and air-exposed guinea pigs appeared normal.

Daily Postexposure Animal Observations

Perinasal and perioral wetness, noted 30 to 60 min following MIC exposure, developed into encrustations. Also, breathing difficulties were present in all rats exposed to 20.4 or 10.5 ppm. There were fewer rats with clinical abnormalities during the second postexposure week than the first postexposure week. No signs of toxic effect in males or females of the 2.4, 1.0, or 0 ppm groups were found.

All male and female mice exposed to 20.4, 10.5, or 5.4 ppm of MIC had decreased activity and were hypothermic during the first postexposure week. Mice exposed to 20.4 ppm of MIC were also ataxic. As was the case with rats, the number of mice with clinical abnormalities lessened during the second postexposure week.

Daily postexposure observations for male and female guinea pigs were minimal. Periocular encrustations, partially closed eyes, and decreased activity were the clinical signs seen in only one female guinea pig exposed to 5.4 ppm of MIC. Male and female guinea pigs of the 2.4, 1.0, and 0 ppm exposure groups were normal in appearance and behavior.

Mortality

The time and frequency of mortality for the rats, mice, and guinea pigs are given in Table 2. Partial to complete group mortality occurred in all species exposed to 20.4, 10.5, or 5.4 ppm of MIC. No deaths occurred in animals exposed to 2.4, 1.0, or 0 ppm of MIC. The most common time of death was the first 48 hr postexposure, in which 55% of all animal deaths occurred. Delayed deaths were most common during postexposure days 8 through 10, in which 22% of all animal deaths occurred. The sex of the animal did not appear to be a factor affecting time or incidence of mortality; therefore, the number of deaths of males and females per concentration level was combined in order to calculate the LC_{50} value for each species. Table 3 presents the LC_{50} values and 95% confidence limits for the study.

The 6-hr LC_{50} value in mice was approximately twice that of the rats or guinea pigs, suggesting mice were less susceptible to acute MIC exposures. A partial explanation for this result may be the greater ability of the mouse to decrease respiratory rate by reflex sensory irritation stimulation compared to the rat or guinea pig. The net result of this decrease in respiratory rate would be a decrease in minute volume and subsequently a decrease in the effective dose inhaled by the mouse. This reflex mechanism has been observed in mice exposed to various isocyanates, including MIC (*14*) or diisocyanates (*15*), and is a common finding with respiratory irritants (*16*). Differences between species as to the magnitude of decrease in respiratory rate following inhalation of an irritant vapor have been observed with formaldehyde (*17*).

Body Weights

The body weights for male and female rats exposed to 20.4, 10.5, or 5.4 ppm of MIC were significantly ($p < 0.001$ lower than rats of the control group throughout the postexposure period. The male rats of the 2.4 ppm exposure group had statistically significant ($p < 0.05$)

Table 2. Time and incidence of mortality for F344 rats, B6C3F1 mice, and Hartley guinea pigs following a single 6-hr exposure to methyl isocyanate vapor

MIC concentration, ppm	Sex and species	No. dying during exposure	No. dying on postexposure day											Total dying before sacrifice
			1	2	3	4	5	6	7	8	9	10	11–14	
20.4	Male	0	1	2	2	0	0	0	0	0	0	0	1	6/6
10.5	rats	0	0	1	0	0	0	0	0	0	1	1	0	3/6
5.4		0	0	0	0	0	0	0	0	0	4	0	1	5/6
2.4		0	0	0	0	0	0	0	0	0	0	0	0	0/6
1.0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
20.4	Female	0	1	5	—	—	—	—	—	—	—	—	—	6/6
10.5	rats	0	1	0	0	0	0	1	0	0	1	0	0	3/6
5.4		0	0	0	0	0	0	0	0	2	0	3	0	5/6
2.4		0	0	0	0	0	0	0	0	0	0	0	0	0/6
1.0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
20.4	Male	0	1	3	1	0	1	—	—	—	—	—	—	6/6
10.5	mice	0	0	2	0	0	1	1	0	0	0	0	0	4/6
5.4		0	0	0	0	0	0	0	0	0	0	0	0	0/6
2.4		0	0	0	0	0	0	0	0	0	0	0	0	0/6
1.0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
20.4	Female	0	0	1	1	0	1	0	0	1	0	0	0	4/6
10.5	mice	0	0	0	0	0	0	0	0	0	0	0	0	0/6
5.4		0	0	1	0	0	0	0	0	0	0	0	0	1/6
2.4		0	0	0	0	0	0	0	0	0	0	0	0	0/6
1.0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
10.5	Male	0	5	0	1	—	—	—	—	—	—	—	—	6/6
5.4	guinea	0	0	1	0	0	0	0	0	0	0	0	0	1/6
2.4	pigs	0	0	0	0	0	0	0	0	0	0	0	0	0/6
1.0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
10.5	Female	0	6	—	—	—	—	—	—	—	—	—	—	6/6
5.4	guinea	0	0	2	1	0	0	0	1	0	0	0	0	4/6
2.4	pigs	0	0	0	0	0	0	0	0	0	0	0	0	0/6
1.0		0	0	0	0	0	0	0	0	0	0	0	0	0/6
0		0	0	0	0	0	0	0	0	0	0	0	0	0/6

Table 3. Six-hour LC_{50} values for methyl isocyanate.

Species	LC_{50}, ppm	95% confidence interval, ppm[a]
Rats	6.1	4.6–8.2
Mice	12.2	8.4–17.5
Guinea pigs	5.4	4.4–6.7

[a] Male and female data combined.

decreases in body weight or body weight gain throughout the postexposure period; however, the females of this group had lower body weights following the first postexposure day only. Partial recovery from body weight losses was observed in the 2.4 ppm-exposed rats only. Body weight means for rats of the 1.0 ppm exposure level were similar to the control values throughout the study.

Statistically significant ($p < 0.05$) decreases in body weight were found throughout the postexposure period for male and female mice exposed to 20.4, 10.5, 5.4, and 2.4 ppm of MIC. Surviving male mice of all groups exhibited some body weight recovery, demonstrated by the slight increase in weight on postexposure day 14 when compared to the weight obtained on postexposure day 7. Only female mice of the 1.0 ppm exposure group had statistically significant ($p < 0.05$) decreases in body weight on postexposure days 1 and 14.

Male and female guinea pigs of the 5.4 ppm exposure group had statistically significant ($p < 0.01$) depressions in body weight on postexposure day 1. Survivors of this group recovered from the initial loss in weight. There were no statistically significant differences in body weight in the 2.4 and 1.0 ppm-exposed guinea pigs when compared to control values.

Pathology

Rats. The gross lesions of biological significance observed in the rats that died following exposure were dark red to brown material encrusted around the mouth, nares, and eyes, as well as on the forepaws, with congestion of the lungs. Histologic changes were present throughout the respiratory tract and consisted of congestion, epithelial necrosis, and inflammation of the

nasal tissues, larynx, trachea, and lungs. Edema was also present in the lungs (Figs. 1 and 2). No rats survived following exposure to 20.4 ppm.

Fifty percent of the rats exposed to 10.5 ppm died during the postexposure observation period (Table 2). Histologic findings in those that died were similar to findings in the 20.4 ppm exposure group. Additional changes developing during a longer survival period included squamous metaplasia involving the upper airways and submucosal fibroplasia in the bronchi and bronchioles of the lung (Fig. 3). The rats exposed to 10.5 ppm that survived until the end of the 14-day observation period had more severe submucosal fibroplasia in the airways and interstitial fibrosis in some lung lobes (Fig. 4). By the end of the observation period, epithelial regeneration was evident in the upper respiratory tract as well as lower in the lungs (Fig. 5). Fibrinous and/or purulent pneumonia, as well as alveolar histiocytosis, were occasionally present and were localized in distribution.

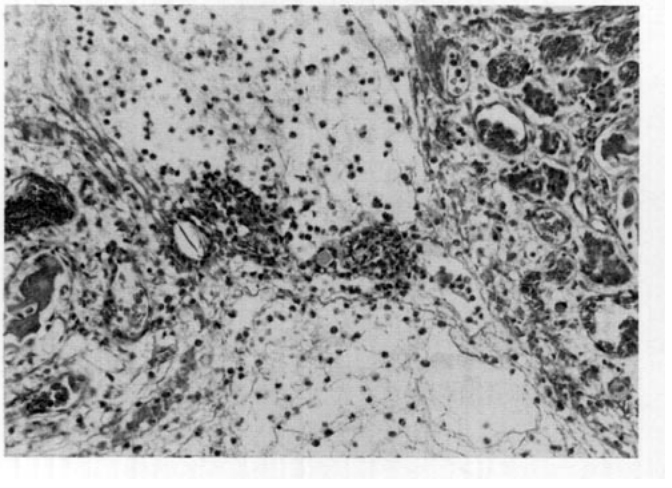

FIGURE 1. Fibrinous rhinitis, congestion, and necrosis in the anterior nasal cavity of a male rat exposed to 20.4 ppm of MIC. H & E, × 160.

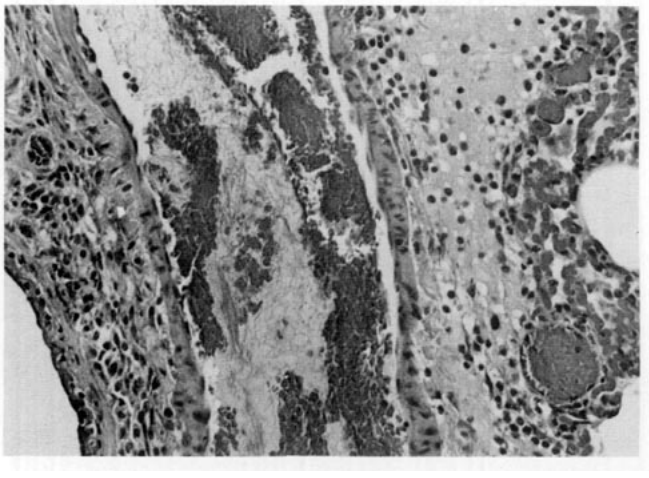

FIGURE 2. Bronchiolar epithelial necrosis, perivascular edema, and interalveolar capillary congestion in the lung of a male rat exposed to 20.4 ppm of MIC. H & E, × 160. (Reprinted courtesy of Academic Press.)

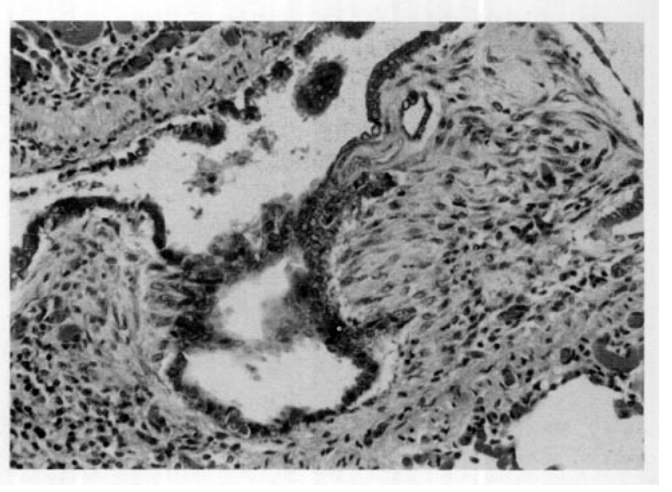

FIGURE 3. Submucosal fibroplasia with epithelial elevation in a bronchiole of a male rat exposed to 10.5 ppm of MIC vapor that died several days following exposure. H & E, × 160. (Reprinted courtesy of Academic Press.)

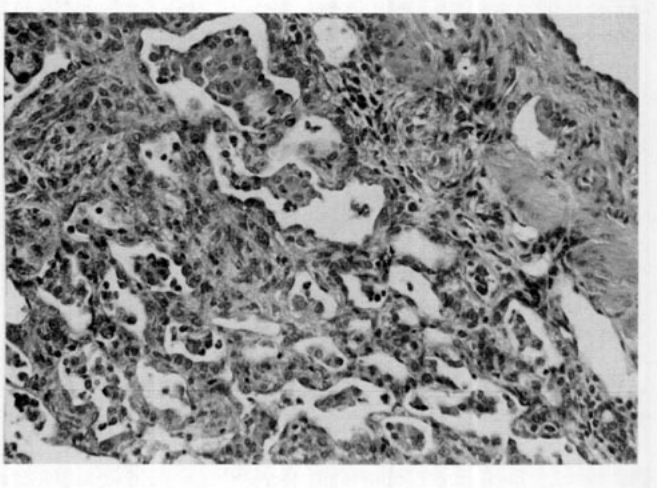

FIGURE 4. Bronchiolar epithelial erosion and interstitial fibrosis in the lung of a male rat that was sacrificed on postexposure day 14 following exposure to 10.5 ppm of MIC. H & E, × 160. (Reprinted courtesy of Academic Press.)

Five of six rats of both sexes died 8 to 14 days following exposure to 5.4 ppm (Table 2). The longer survival of these rats was reflected in respiratory tract lesions, which included epithelial regeneration, squamous metaplasia, and intraluminal airway fibrosis. The two surviving rats from this group sacrificed at the end of the observation period exhibited similar lesions to those observed in the survivors of the 10.5 ppm exposure group.

All rats exposed to 2.4 ppm or 1.0 ppm of MIC vapor for 6 hr survived until sacrifice, as did the controls exposed to room air only. The only change of biological significance observed in some of the males of the 2.4 ppm exposure group was occasional epithelial regen-

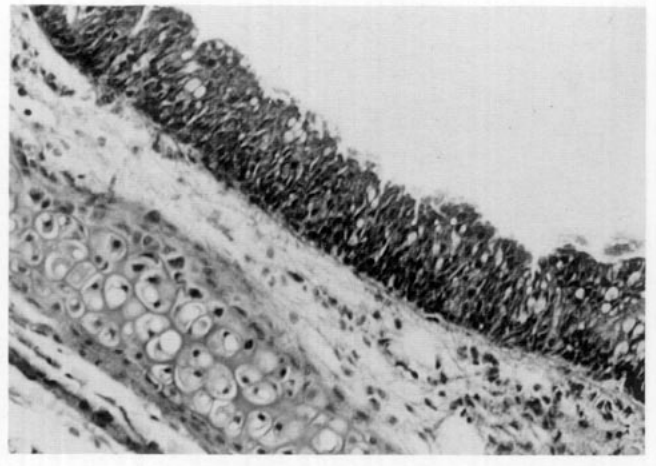

FIGURE 5. Epithelial regeneration in the trachea of a male rat that was sacrificed on postexposure day 14 following exposure to 10.5 ppm of MIC. H & E, × 160.

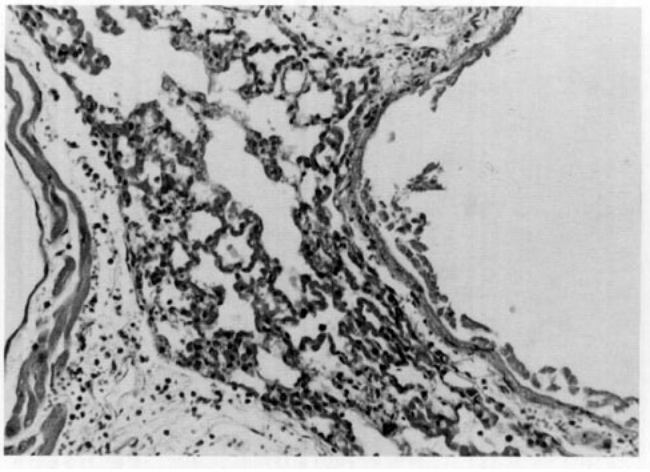

FIGURE 6. Bronchiolar epithelial necrosis and alveolar capillary congestion in the lung of a male mouse that died following exposure to 20.4 ppm of MIC. H & E, × 160. (Reprinted courtesy of Academic Press.)

eration in the larynx, trachea, and bronchi. No lesions were observed in the 1.0 ppm exposure group.

Mice. Mice were more resistant to MIC vapor as evidenced by the fact that fewer mice died following exposure, especially the females (Table 2), and that the gross lesions observed following death were not as severe, particularly with respect to the nature of the perinasal exudate and the number of mice affected. However, the lungs were discolored in all of the mice that died following exposure to 20.4 ppm. The histologic changes observed in these mice consisted of severe congestion, necrosis, and moderate inflammation of the nasal tissues, severe necrosis of the larynx and trachea, and edema, congestion, and bronchiolar epithelial necrosis of the lungs (Fig. 6). Two females survived the 20.4 ppm exposure until sacrifice, at which time no gross lesions were observed. Histologically, the changes consisted of incomplete regeneration of olfactory epithelium and squamous metaplasia of respiratory epithelium in the nasal cavity (Fig. 7), intraluminal airway fibrosis of the trachea and larger pulmonary airways (Fig. 8), as well as interstitial fibrosis, epithelial necrosis, and epithelial regeneration in the lungs.

Only four male mice died following exposure to 10.5 ppm of MIC vapor. Those mice that survived until sacrifice had no gross lesions, and the histologic lesions were similar to those seen in the two mice that survived the 20.4 ppm concentration.

Only one female mouse died following exposure to 5.4 ppm of MIC vapor; this mouse had evidence of necrosis and congestion in the respiratory tract. Nearly all survivors of the 5.4 ppm exposure had lesions in the nasal cavity consisting of incomplete regeneration of olfactory epithelium; occasionally, there was evidence of incomplete regeneration and squamous metaplasia in the respiratory epithelium. These latter changes were ob-

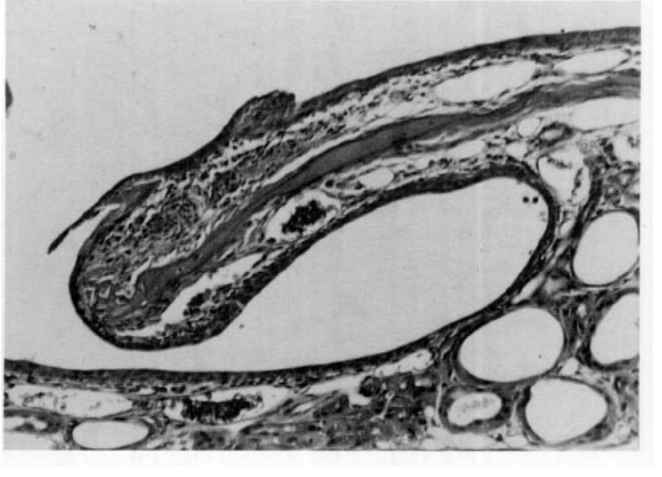

FIGURE 7. Early squamous metaplasia of the turbinate epithelium in the anterior nasal cavity of a female mouse sacrificed on postexposure day 14 following exposure to 20.4 ppm of MIC. H & E, × 160.

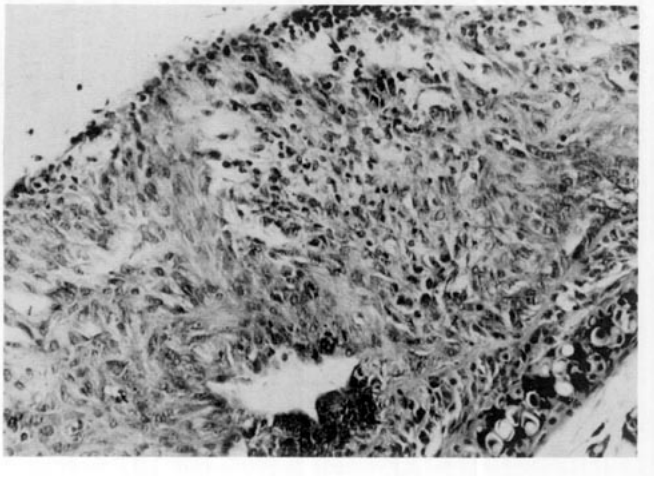

FIGURE 8. Marked submucosal fibroplasia in the trachea of the same mouse described in Fig. 7. H & E, × 160. (Reprinted courtesy of Academic Press.)

served in the most anterior section of the nasal cavity. Intraluminal airway fibrosis in the bronchioles was more severe and of greater prevalence in the male mice than in the females. Epithelial regeneration was evident throughout the respiratory passages in most mice.

No deaths occurred in mice exposed to 2.4 ppm or 1.0 ppm of MIC vapor, and no significant gross lesions were observed. Occasionally, a mouse from the group exposed to 2.4 ppm had evidence of epithelial atrophy or regeneration in the upper respiratory passages down to and including the trachea. No lesions of biological significance were found in the lungs.

Guinea Pigs. All but one guinea pig exposed to 10.5 ppm of MIC vapor died the day following exposure. Perinasal encrustation with reddish-colored material was noticed grossly in all of the males, but only in one of the six dead females. The lungs were grossly reddened in all guinea pigs that were exposed to this concentration. Histologic findings consisted of marked to severe congestion, necrosis, and rhinitis of the nasal cavity (Fig. 9), marked necrosis of the larynx and trachea (Fig. 10), and congestion, hemorrhage, edema, hyaline membrane formation, and necrosis of the lungs (Fig. 11). A striking lesion in the lungs was the nearly complete obliteration of the bronchioles by exfoliated epithelial cells (Fig. 12). This obliteration appeared to include a narrowing of the lumen due to smooth muscle contraction, unlike the passive collection of debris in the lumina of rodent airways. The one male guinea pig that survived 2 days longer than the others had evidence of squamous metaplasia in the larynx and trachea, and marked pneumonia in the lungs.

One male and four female guinea pigs died following exposure to 5.4 ppm of MIC vapor. Only two of the four females had evidence of nasal discharge, but all had gross lesions in the lungs. Histologic findings were similar to those observed in the higher exposure group, although somewhat less severe, especially with respect to the upper airways.

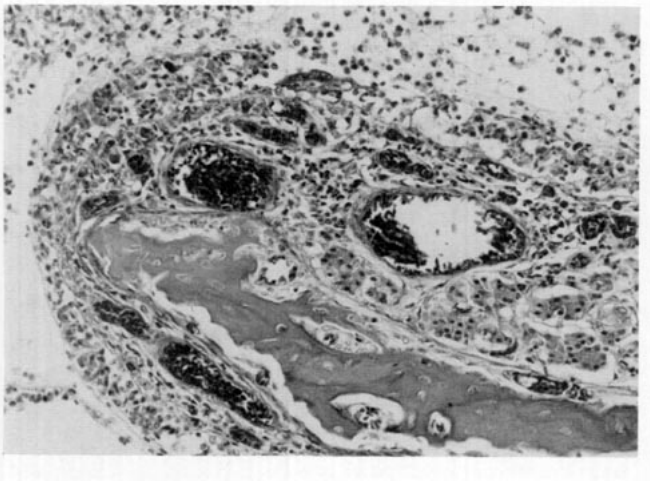

FIGURE 9. Congestion, necrosis, and inflammation of a turbinate from the anterior nasal cavity of a male guinea pig that died following exposure to 10.5 ppm of MIC. H & E, × 160. (Reprinted courtesy of Academic Press.)

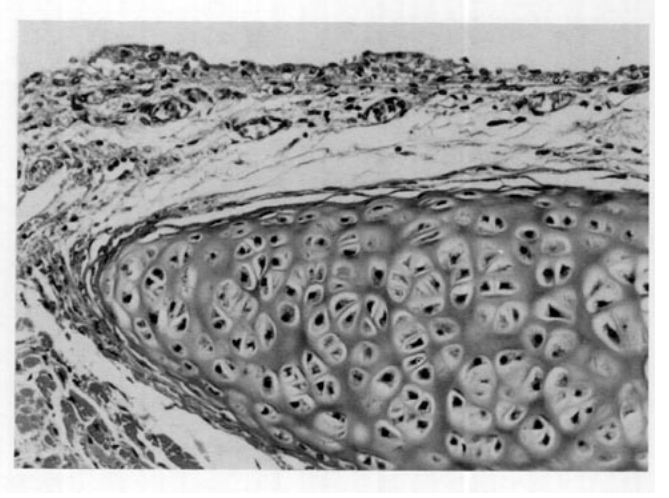

FIGURE 10. Tracheal epithelial necrosis of the same guinea pig described in Fig. 9. H & E, × 160. (Reprinted courtesy of Academic Press.)

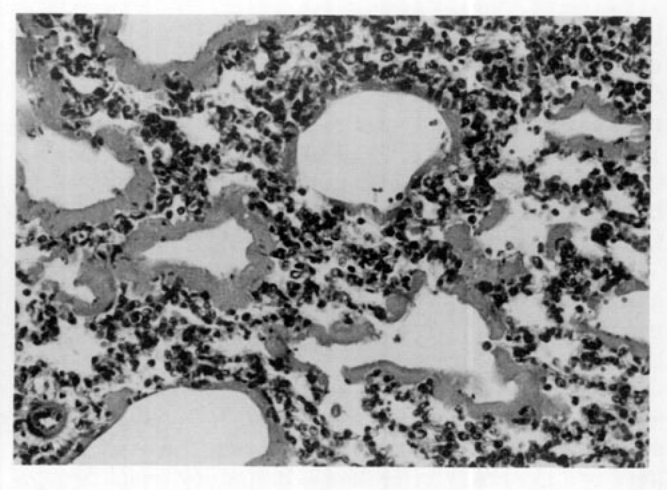

FIGURE 11. Hyaline membrane formation and inflammation in the lungs of the same guinea pig described in Fig. 9. H & E, × 160. (Reprinted courtesy of Academic Press.)

The guinea pigs that survived the 5.4 ppm exposure had no gross lesions at the time of sacrifice. The histologic lesions observed in the respiratory tract included small intraepithelial microabscesses within the respiratory mucosa, and occasionally, incomplete regeneration of olfactory epithelium and rhinitis. Epithelial regeneration in the larynx and trachea was observed in about one-half of the animals. A number of pulmonary changes were present, including bronchopneumonia, interstitial pneumonia, and alveolar histiocytosis, in addition to epithelial regeneration in the bronchi and bronchioles.

No guinea pigs died following exposure to 2.4 or 1.0 ppm of MIC vapor. Only one female exposed to 2.4 ppm had discolored lungs at the time of necropsy. The histologic lesions of the nasal cavity of biological impor-

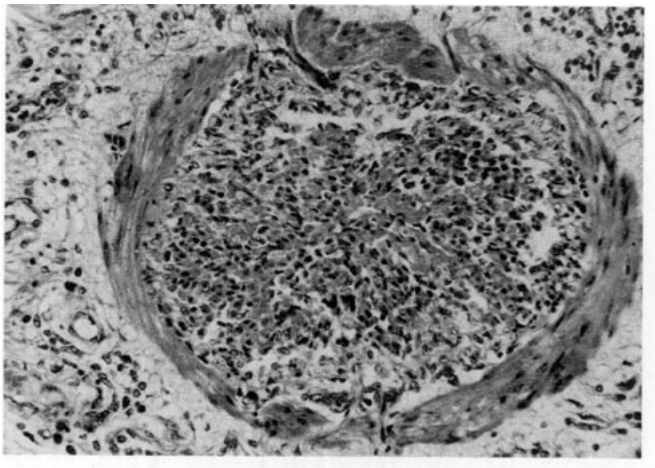

FIGURE 12. Bronchiolitis obliterans in the lung of the same guinea pig described in Fig. 9. H & E, × 160. (Reprinted courtesy of Academic Press.)

tance in these two exposure groups consisted of degeneration of olfactory and/or respiratory epithelium, observed in three males and one female from the 2.4 ppm exposure group, and in one of six males from the 1.0 ppm exposure group. Some evidence of squamous metaplasia, epithelial necrosis, and regeneration of the larynx or trachea was present in two of the males from the 2.4 ppm exposure group.

In the lungs, epithelial regeneration of the bronchi and bronchioles was observed in two of six females exposed to 2.4 ppm and in one of the six males exposed to 1.0 ppm of MIC vapor.

The greater sensitivity of guinea pigs at the 10.5 ppm exposure concentration may be due to the greater amount of MIC vapor that gets into the lungs. Rats and mice are generally considered obligatory nasal breathers; therefore, the MIC vapor usually passes through the nasal cavity before inspiration to the lungs. However, guinea pigs are optional oral breathers when exposed to severe respiratory irritants. In the present study, guinea pigs began to mouth breathe earlier than rats or mice during MIC exposure; therefore, the "scrubbing" mechanism of the nasal cavity was circumvented, probably resulting in increased concentrations of MIC reaching the lungs. The comparative damage to the nasal tissues and lungs between the species would support this hypothesis in that the nasal tissues were not affected as diffusely or severely in the guinea pig as in the rat, and in that bronchiolitis obliterans was observed in the guinea pigs' lungs but not in the other two species. Other lesions observed in the lungs more frequently and with greater severity in guinea pigs were edema and hyaline membrane deposition. Some evidence of pulmonary damage was also observed at lower exposure concentrations in the guinea pig than in the rat or mouse.

In a comparative study between rats and guinea pigs, guinea pigs were more sensitive to inhalation of ozone alone or in combination with sulfuric acid aerosol and developed lesions in the terminal bronchioles consisting of epithelial hypertrophy and hyperplasia (*18*). Sulfuric acid aerosol led to obliteration of the terminal bronchioles in guinea pigs as a result of desquamation of terminal bronchiolar epithelium and reflex airway constriction; this constriction could be effectively blocked by atropine (*19*). These studies suggest that the guinea pig may respond to MIC as it does to sulfuric acid, with desquamation of terminal bronchiolar epithelium, manifested in the present study as bronchiolitis obliterans.

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