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Two-Hour Methyl Isocyanate Inhalation Exposure and 91-Day Recovery: A Preliminary Description of Pathologic Changes in F344 Rats

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The accidental release of methyl isocyanate (MIC) in Bhopal, India, was reportedly responsible for the deaths of more than 2,000 people. To study the pathology of acute inhalation exposure to MIC, the tissues of male and female Fischer 344 rats were evaluated immediately after a single 2-hr exposure to 0, 3, 10, or 30 ppm MIC, and through day 91. Early gross pathologic changes in the 30 ppm-exposed rats included a reddish white encrustation around the mouth and nose, a small thymus, and distension of the gastrointestinal tract with gas. Lungs (middle and median lobes) showed consolidation and hemorrhage and failed to deflate when the chest cavity was opened. Microscopic changes in the upper respiratory tract 3 hr after exposure included marked erosion and separation of olfactory and respiratory epithelia from the basement membrane with accumulation of serofibrinous fluid. On day 1, acute inflammation and fibrinopurulent exudate partially blocked the nasal passages. Epithelial cells had sloughed from the nasopharynx, trachea, bronchi, and major bronchioles, leaving the basement membrane covered with fibrin and exudate. Granulomatous inflammation and intraluminal fibrosis of the airways were observed by day 3, with increased intraluminal fibrosis by day 7. Lower airways became blocked by exfoliated cells, mucous plugs, and/or intraluminal fibrosis. Damage to the lung parenchyma, even at lethal concentrations, was limited to moderate inflammation. Intraluminal fibrosis, mild bronchitis and bronchiolitis, and mucous plugs persisted throughout the 91-day study. These changes could account for evidence of obstructive lung disease detected in pulmonary function studies in companion studies. Evidence of direct injury to nonrespiratory tissues was not found; pulmonary injury and associated respiratory obstruction appeared sufficient to cause both early and delayed deaths.

Introduction

The accidental release of methyl isocyanate (MIC) from an agricultural chemical plant was reportedly responsible for the deaths of more than 2,000 people in the city of Bhopal, Madhya Pradesh, India, on the night of December, 2, 1984 (*1,2*). Approximately 40 tons of material was released into the atmosphere from an MIC storage tank, causing the deaths of people and animals in the vicinity of the plant (*3*). Initial symptoms included severe irritation of the nose and throat, photophobia, intense lacrimation and burning sensations in the eyes, coughing, chest pain, and choking sensation. Some victims became unconscious and died within minutes of the exposure. Others suffered nausea, excessive salivation, giddiness, tremors, incoordination, and limb paralysis. Preliminary follow-up studies of survivors indicated persistent pulmonary injury, including evidence of obstructive and restrictive changes in airways (*3*).

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A similar spectrum of respiratory and neurological symptoms was seen in firemen accidentally exposed to a related isocyanate, toluene diisocyanate (TDI), in 1967 (*4*). In ensuing studies, respiratory symptoms were still observed 44 months after the exposure to TDI (*4*). In other long-term studies of workers in a polyurethane plastic manufacturing plant, low level exposures (0.001 ppm) to isocyanates were not shown to be associated with chronic respiratory symptoms or effects on ventilatory capacity (*5*).

MIC is used primarily in the manufacture of carbamate pesticides, including aldicarb, carbaryl, carbofuran, and methomyl (*2*). MIC is a flammable liquid, has a boiling point of 39°C, reacts with water and a variety of functional groups (*6*), is corrosive to skin and mucous membranes, and causes severe irritation and corneal necrosis when placed in the eye of rabbits (*7,8*). MIC is both a sensory and pulmonary irritant and causes extensive necrosis, hemorrhage, epithelial erosion, and alveolar damage in rats exposed for 1 hr at the concentration of 1 mg/L (*9*). The oral LD_{50} was reported as 71

mg/kg in rats and 120 mg/kg in mice; by inhalation, the 4 hr LC_{50} was reported as 5 ppm in rats (*8*). In our studies, an approximate 2 hr LC_{50} was found to be between 20 to 25 ppm for rats, and 25 to 30 ppm for mice (*10*). Male mice and male rats were somewhat more sensitive to the acute lethal effects than females. Earlier reports attributed death of rats and mice following exposure to MIC to acute pulmonary edema and bronchospasm, with symptoms of asthmatic breathing and pneumonia in survivors (*7*). Similar effects were seen with other isocyanates (*11*). While there are many reports of toxicities associated with exposure to isocyanates (*4,11–16*), no studies are available on the long-term health effects related to exposure of man or animals to MIC. The current series of studies undertaken at the National Institute of Environmental Health Sciences was designed to examine both acute and long-term effects of inhalation exposures to MIC in animals. This paper is a preliminary report of gross and histopathologic evaluations of rats exposed to 0, 3, 10, or 30 ppm MIC for a single 2-hr period and followed for up to 91 days. A more detailed report is planned following peer review of the pathology findings.

Materials and Methods

Methyl isocyanate was obtained from Union Carbide Corp., Research Triangle Park, NC, and determined to be greater than 99% pure by gas chromatography with flame ionization detection. Animals were exposed in 1330 L, stainless steel chambers. Complete details of vapor generation and monitoring and safety aspects have been described by Adkins et al. (*17*).

Four- to six-week-old, male and female F344 rats were obtained from Charles River Breeding Laboratories, Inc. (Kingston, NY, or Portage, MI). Rats were quarantined for 2 to 3 weeks prior to exposure. Rats were randomized to exposure groups on the basis of body weight. Four male or female rats were housed in stainless steel cages during exposures and in polycarbonate cages after exposures. The details of the animal care have been previously described (*10*).

Rats were subjected to a single 2-hr exposure to 0 (control), 3, 10, or 30 ppm MIC. Within 3 hr of the exposures (day 0), and again on days 1, 3, 7, 14, 28, 49, and 91 postexposure, five predesignated rats from each group and sex were killed by IP pentobarbital sodium overdose (Nembutal sodium solution, Abbot Laboratories, North Chicago, IL). Immediately after euthanasia, all rats were subjected to a complete necropsy evaluation. Necropsies were also performed on dead and moribund rats, and these results will be reported separately. All tissues were fixed in buffered (pH 7.0), 10% formalin. The tissues that were routinely examined microscopically included the nasal passages (three levels), nasopharynx, trachea at the level of the thyroid, parathyroid, esophagus, peribronchial lymph nodes, brain, kidneys, thymus, spleen, heart, glandular and nonglandular stomach. On days 7 and 91, in addition to the routine tissues, the following also were examined: pancreas, salivary glands, manibular lymph nodes, adrenal glands, pituitary gland, larynx, six levels of intestinal tract, mesenteric lymph nodes, abdominal skin, mammary gland, urinary bladder, femur including bone marrow, and sex organs (males: testis, epididymis, seminal vesicles, prostate, and preputial glands; females: ovaries, uterus, and clitoral glands).

The head was removed at the atlanto-occipital joint, and the nasal cavity was retrogradely infused through the nasopharynx with 2 to 5 mL of formalin to assure adequate fixation. The lung was infused with 4 to 10 mL of formalin through the trachea, which was then tied off, and the lung was immersed in formalin. After fixation of the head, excess skin, fascia, and muscle were removed, the head was placed in decalcifying solution (American Scientific Products, McGraw Park, IL). Following overnight decalcification, transverse sections were taken just posterior to the incisor teeth; midway between incisors and first molar; and middle of the second molar. Trimmed tissues were embedded in paraffin, sectioned 6 μm thick, and stained with hematoxylin and eosin for light microscopy.

Results

As noted previously (*10*), male rats were more prone than females to early death following 30 ppm MIC exposure. In the current study, only four of eighty-eight males exposed to 30 ppm survived to day 91. The first mortality in male rats was noted on day 1 and in females on day 7. No unscheduled deaths occurred in the 0, 3, or 10 ppm groups. Complete descriptions of in-life observations (body weights, clinical signs, organ weights, and clinical pathology and hematology) for these animals have been reported (*10*).

Gross Pathologic Findings

Pathologic changes were more severe in the 30 ppm group than in the 10 or 3 ppm rats. Reddish white encrustations were observed around the mouth, nose, and eyes of dead and moribund rats. The middle and median lobes of the lung were commonly consolidated in both females and males. Petechiae and ecchymoses were observed in the lung of rats in the 30 ppm groups. Consolidated lobes often did not inflate when perfused with formalin, while other lobes without gross lesions (primarily 30 ppm concentration group) remained inflated when removed from the thoracic cavity. In the rats that died following exposure, the stomach was usually devoid of food, and the gastrointestinal tract was distended with gas. There was a general lack of body fat in high exposure concentration animals as compared to controls. These findings corresponded with lower body weights in these animals (*10*). The thymus was smaller in 30 ppm rats than in controls. Minimal to moderate edema, congestion in the region of the nasopharynx in moribund rats, and fibrin clots attached to the hard palate and pharyngeal regions were seen in a few 30 ppm rats.

Microscopic Findings

Treatment-related microscopic changes were restricted to the upper and lower respiratory tracts and the thymus. Thymic atrophy in 30 ppm female rats was observed only on days 7 and 14. Karyorrhexis and pyknosis of thymocytes with thymic atrophy (primarily 30 ppm concentration) was found on days 3 through 28 in the males. Males exposed to 30 ppm MIC did not survive beyond day 28.

Microscopic changes in the respiratory tract of MIC-exposed rats were similar in both sexes; however, the lesions were sex and concentration dependent, being more severe in males and at 30 ppm. The stratified squamous epithelium of the nasal passage did not appear to be affected. In general, the lesions of the respiratory epithelia were more severe in anterior and median parts of the nasal pasage compared to the lateral and more posterior parts.

Lesions observed at the 3 ppm concentration were mild and essentially resolved by day 14. Two hours after exposure, necrosis of the respiratory epithelium of the trachea was seen, characterized by epithelial cells with eosinophilic cytoplasm and pyknotic nuclei, with loss of intercellular attachments. By postexposure day 1, the respiratory epithelium in the nose, trachea, and occasionally the bronchi was flattened and lacked cilia. There wre increased cell numbers by postexposure day 7, but by day 14, the epithelia appeared essentially normal.

The results of the 10 ppm and the 30 ppm exposures are presented by time after exposure, with emphasis on the nature of the lesions. Sex and concentration distinctions are described only when these differences were striking.

Day 0. Within 3 hr of exposure, necrosis of both respiratory and olfactory epithelium occurred in the nasal cavity, with erosion in the more severely affected animals. Multifocal necrosis and erosion also involved trachea, mainstem bronchi, secondary bronchi, and even bronchioles in several animals. The earliest indication of significant devitalization of the respiratory epithelium of the airways was basilar cytoplasmic vacuolization, separation from adjacent cells, and detachment from the basement membrane. Increased secretory product or transudate was present in the lumen of the nasal passages and lower airways.

Days 1 and 3. Acute inflammatory cells invaded the lamina propria of airways, and suppurative inflammation intermixed with desquamated epithelial cells was present in the lumina of the nose, trachea, bronchi, and bronchioles. This exudate was fibrinous in several animals and tended to collect within the angles of the turbinates, the maxillary sinus and dorsal meatus in the nose, at the bifurcation of the trachea, in the mainstem bronchi, and at the branch points of bronchi or in bronchioles. Ulceration of the bronchi was so severe in some 30 ppm males that only fibrin casts covered the lamina propria, and blood filled the airways. By contrast, female rats had severe erosion but much less ulceration. When necrosis and sloughing extended the full length of the airway down to the alveolar duct, atelectasis of the lobe often occurred. By day 3, the inflammation became granulomatous with the presence of macrophages and fibrosis of the submucosa and lamina propria of the bronchi and bronchioles. Where ulceration was severe, little regeneration occurred during this time period.

Day 7. In the nasal passages, regeneration of respiratory epithelium was progressing to hyperplasia, except in some animals where regeneration was still at an early stage. The olfactory epithelium was only a few layers of flat to cuboidal cells. Where ulceration and/or inflammatory exudate was present, the respiratory epithelium regenerated more slowly and adhesions between respiratory surfaces occurred. In the trachea, especially near the bifurcation, fibrinous exudate had organized into mural and intraluminal fibrosis covered by regenerative epithelium. Occasionlly, this intraluminal fibrosis partially obstructed the trachea and bronchi, often trapping remnants of respiratory epithelium. While some areas of ulceration were partially epithelized, large areas of lamina propria in the trachea and mainstem bronchi remained exposed. Intraluminal fibrosis was most extensive at the junctions of the lobar and segmental bronchi and the broncho-bronchiolar junction but was also noted in some bronchioles. In a few animals ulceration extended down to the bronchioles with minimal regeneration.

Day 14. In the nasal passages, adhesions between adjacent respiratory epithelial surfaces were present. Olfactory epithelial regeneration was characterized by a multilayered and disorganized epithelium, with replacement by respiratory epithelium on the medial aspect of the ethmoturbinates and the dorsal meatus. In the trachea of a few animals, prominent intraluminal fibrosis and regeneration were present, especially near the tracheal bifurcation. Intramural cysts formed by trapped respiratory epithelium were also observed. In the lung and mainstem bronchi, the respiratory epithelium contained areas of regeneration, hyperplasia, and occasionally, squamous metaplasia. The bronchi and bronchioles also had ulcers, intraluminal fibrosis, and respiratory epithelium trapped in the submucosa and mucous exudate, especially near the terminal bronchioles. In some cases, pulmonary atelectasis appeared to be associated with the mucous plugs. There was also an increase in suppurative inflammation throughout the lung on day 14. Multifocal interstitial fibrosis was occasionally found in association with the areas of inflammation.

Day 28. A few lesions were found in the nasal passages and were mainly in the 30 ppm animals. These changes consisted of incomplete olfactory regeneration and respiratory epithelial metaplasia of the olfactory mucosa. The sex difference in severity of the pulmonary lesions was quite striking on day 28. Female rats at the 10 ppm concentration had mild intraluminal fibrosis in the bronchi and bronchioles with mucous exudate in the bronchioles, while males exposed to this concentration had more severe lesions. At 30 ppm, the females had

some ulceration of the respiratory epithelium of the bronchi and bronchioles with advanced regeneration. In the males, epithelization of the airways was incomplete. Intramural cysts and trapped epithelium were present in the airways of both males and females.

Day 49. By this time, all the male rats in the 30 ppm group had died. Female rats exposed to the 30 ppm concentration continued to have hyperplastic and regenerative lesions in the respiratory epithelium of the nasal passages, and a single animal had minimal adhesions. The olfactory epithelium had regenerated, except where respiratory epithelial metaplasia was present. In the bronchi and bronchioles of the 30 ppm females, hyperplasia, regeneration, minimal ulceration, intraluminal fibrosis, mucous exudation, and intramural cyst formation were present. Inflammation was present in the airways, extending to the alveoli. In the 10 ppm male rats, hyperplasia and regeneration of the respiratory epithelium with mucous exudate in the bronchi, bronchioles, and occasionally in the alveoli were found. The nasal cavity and trachea of male and female rats at 10 ppm were essentially normal.

Day 91. Minimal focal respiratory epithelial hyperplasia in the nasal passage was present in males exposed to 10 ppm MIC. Minimal to mild hyperplastic and regenerative respiratory epithelium was still present in the bronchi. The severity of the inflammatory airway lesions had decreased, but some mucous exudate was present. Only two 30 ppm female rats survived to 91 days. In these animals, hyperplasia, regeneration, and minimal focal ulceration of the respirat epithelium of the airways were found with intraluminal bronchial fibrosis and intra-alveolar mucous exudate.

Discussion

The purpose of these investigations was to characterize acute injury resulting from exposure of rodents to lethal and sublethal concentrations of MIC, to determine whether the primary injury was limited to the respiratory tract, and to examine the persistance of any pulmonary effects. The 2-hr exposure duration was chosen to mimic the period of exposure that occurred in Bhopal, India, but some distinct differences exist between these experiments and the accident that should be kept in mind. The experimental exposures were constantly controlled concentrations of pure MIC in air, whereas the concentration in Bhopal and the exact contents of the vapor cloud are unknown. In the controlled exposures, our experiments may have underestimated acute deep lung (alveolar) injury, although it is probable that significant injury to terminal respiratory units is incompatible with survival. The present experimental design probably relates more to injurious processes associated with acute MIC exposure leading to delayed deaths or persistent pulmonary damage in humans.

In agreement with earlier reports (*7–9*), we found exposure of rats to MIC resulted in irritation and corrosive injury of the epithelial tissues lining the nasal passages, larynx, pharynx, trachea, bronchi, and bronchioles. The earliest lesions were necrosis and exfoliation of the olfactory and respiratory epithelia of the airways, which was followed by an influx of serofibrinous fluid. The presence of exudate and necrotic cellular debris in the airway lumen may have been responsible for early deaths from respiratory insufficiency. In animals that survived the initial insult, inflammation and fibrin were present in the airways.

The pathogenesis of intraluminal fibrosis appeared to be an initial insult to the surface epithelium followed by degeneration, necrosis, and erosion of respiratory epithelia, with fibrin deposition over the denuded basement membrane. The fibrin casts serve as a meshwork for the migration and replication of fibroblasts, leading to collagen production and, finally, epithelization of the fibrous connective tissue. The intraluminal fibrosis consisted of two tissue components, a fibroblast core covered with respiratory epithelium.

Intraluminal fibrosis was observed as early as day 7 of postexposure and persisted in the bronchioles of surviving rats. By day 91, there apeared to be maturation and contraction of the fibrous connective tissue as occurs in other types of scar formation. The lungs in the 30 ppm rats often remained inflated when removed from the thoracic cavity. Histopathologic examination showed that inflammatory components, mucus, and intraluminal fibrosis often blocked the airways. These acute and chronic pathologic changes in the airways and the lung parenchyma probably contributed to the obstruction of airflow and loss of compliance in the lung as reflected in alterations of pulmonary function tests found in these rats (*18*).

Further detailed descriptions of injury and healing of the tissues of the nasal cavity can be found in Uraih et al. (*19*), and similar pathologic changes in mice exposed to MIC are reported by Boorman et al. (*20*) and in guinea pigs, rats, and mice by Fowler and Dodd (*21*).

No evidence of primary injury to nonrespiratory tissues was found in the current studies. Thymic atrophy observed early following exposure appeared reversible and was probably secondary to stress and the poor nutritional status of the exposed animals (*10*). Distension of the gastrointestinal tract may have been caused by swallowing air during periods of respiratory distress. The lack of significant eye injury in these animals has been reported by Gupta et al. (*22*).

In summary, significant acute injury following a single 2-hr exposure of rats to MIC was confined to the respiratory tract. Early deaths were probably due to the acute necrotizing action of the chemical, which led to epithelial sloughing and serofibrinous effusion from the nasal cavity to the level of the bronchioles. Survivors developed inflammation with persistent obstructive scarring and mucus accumulation in the small airways. Such changes would be sufficient to account both for deaths which occurred after the first week postexposure and for diminished pulmonary function in survivors. The 2-year studies in progress in rats and mice may provide additional information on the long-term effects of acute MIC exposure.

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