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Effect of Methyl Isocyanate (MIC) Gas On the Eyes of Fischer 344 Rats

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The accidental release of methyl isocyanate gas in Bhopal, India, was reported to cause temporary blindness and other eye injuries in many of the exposed people. Methyl isocyanate (MIC) is known to be corrosive and to irritate intact skin and mucous membranes, but little is known about the extent of ocular damage incurred during exposure to its vapors. The eyes of male and female Fischer 344 rats were evaluated immediately after a 2-hr exposure to 0, 3, 10, or 30 ppm of MIC, and periodically thereafter during a 91-day recovery period. During exposure to 10 ppm and higher concentrations, rats kept their eyes partially closed. Copious lacrimation and occasional frothy nasal discharge were evident. Eyes were examined under ultraviolet light after topical application of sodium fluorescein, and histopathologic examination included lids, cornea, lens, retina, optic nerve, and Harderian gland. There was no significant gross or microscopic evidence of epithelial erosion or ulceration of the cornea, or of adjacent tissues immediately after, or at any time following exposures. No skin irritation was noted. It would appear that the natural protective mechanisms of the eye of rats were adequate to prevent ocular damage at these exposure levels.

Introduction

Severe eye irritation and partial or complete blindness were reported in thousands of people exposed to the material that leaked from a methyl isocyanate storage tank at an agricultural chemical plant in Bhopal, India, on December 3, 1984 (*1*). Clinical signs included lacrimation, photophobia, profuse lid edema and superficial corneal ulceration (*1*). The most frequent symptoms were a burning sensation of the eyes and throat and coughing. These people were located in the area close to the chemical plant. Therapeutic measures used by the Indian physicians included eyewash with normal saline, and local application of homatropine or atropine and antibiotic (*2*). In the weeks following the accident, interpalpebral injection (congestion) and signs of a healing epithelium were evident. Severe lacrimation and photophobia were common complaints, and these symptoms appeared to abate slowly.

Methyl isocyanate has been found to cause severe necrosis when directly applied to the eyes of rabbits (*3*). The low molecular weight ethyl and methyl isocyantes were reported to be skin irritants and could cause permanent eye damage (*4*). Erosion of corneal epithelium of rats was reported when exposed to 65 ppm MIC for 2 hr (*5*). One of the earliest signs of exposure to MIC is eye irritation. Human volunteers exposed to 0.5 ppm of MIC for 10 min all reported eye irritation, lacrimation, and nose and throat irritation, but only one of six could detect an odor (*3*). The odor threshold reported for MIC is 2.1 ppm. Both odor and eye irritation thresholds are poor warning signals, as both are considerably higher than the current threshold limit value of 20 ppb (*6*). As part of our studies on the toxicity of methyl isocyanate, the eyes of Fischer 344 rats were examined following exposure to lethal and sublethal concentrations of MIC. The intent of this study was to determine if eye injury occurred in rats during MIC exposure.

Materials and Methods

The experimental design of this study was described previously (*7,8*). Briefly, specific pathogen-free Fischer 344 rats of both sexes were exposed to 0 (control), 3, 10, or 30 ppm MIC for 2 hr on 3/27, or 4/22/85. One hour following exposure (day 0), five rats per sex and dose were killed by IP injection of sodium pentobarbital solution, and a gross necropsy examination was performed on each rat. After euthanasia of the high dose (30 ppm) and control rats on day 0, 1, 3, and 7, a few drops of fluorescein sodium solution were instilled into both eyes. Excess solution was washed away with physiological saline solution. In several euthanatized control rats, superficial scratches were made in the cornea with a fine needle to induce epithelial damage to the cornea and serve as a positive control for the MIC-exposed rats. The fluorescein-treated eyes of control and 30 ppm-ex-

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posed rats were then examined visually under long wave-length ultraviolet light (366 nm).

The head containing the eyes and associated structures from rats in all exposure groups was fixed in neutral buffered 10% formalin for histopathologic examination. Each eye was sectioned in the dorsoventral pupillary/optic nerve plane to include both eyelids and Harderian glands. Tissues were then embedded in paraffin, and 6-μm thick sections were stained with hematoxylin and eosin. These sections containing cornea, eyelids, lens, retina, optic nerve, and Harderian gland were evaluated microscopically. This procedure was performed on all rats taken for histopathologic evaluation (five rats/sex and dose) on days 0, 1, 3, 7, 14, 28, 49, and 91.

Results

Clinical Observations

The rats began to explore their new surroundings soon after they were placed in the inhalation chamber. After 5 to 15 min, rats were quiet and remained so while concentrations of MIC were brought from 0 to about 10 ppm. When the concentration increased to above 10 ppm, some of the rats became restless and showed irritation by moving their heads with a sharp jerky motion. At concentrations of about 15 ppm, rats were generally restless and occasionally jumped or walked in circles. Grooming activities, and rubbing of the eyes and nose with the paws were frequently observed at these intermediate concentrations. At 20 ppm, rats walked with a low carriage with their noses down. They kept their eyes partially closed, and often shook their heads. As the concentration reached 30 ppm, rats tended to lay flat on their bellies (Fig. 1) and almost all activity ceased.

Later during the exposures, rats began to show copious lacrimation (Fig. 2) and signs of respiratory distress. An occasionally blood-tinged, frothy discharge was observed from the noses of high dose animals (Fig. 3). These rats were generally unresponsive to noise, but did not appear to lose consciousness during the exposure. Occasionally all rats in a cage lay parallel to each other during the exposure.

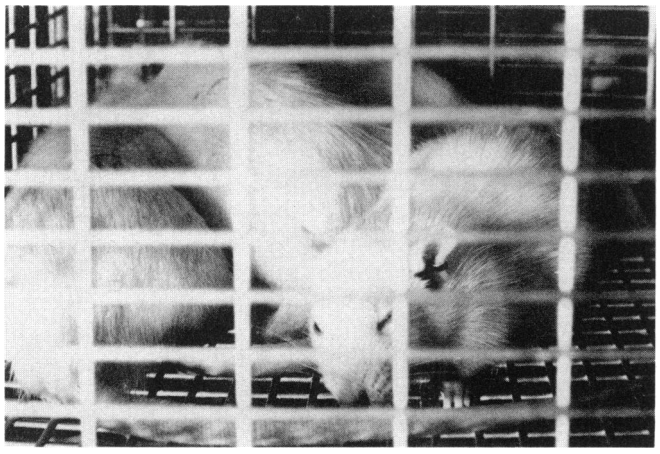

FIGURE 1. Rats in ventral recumbency when the concentration of methyl isocyanate reached 30 ppm.

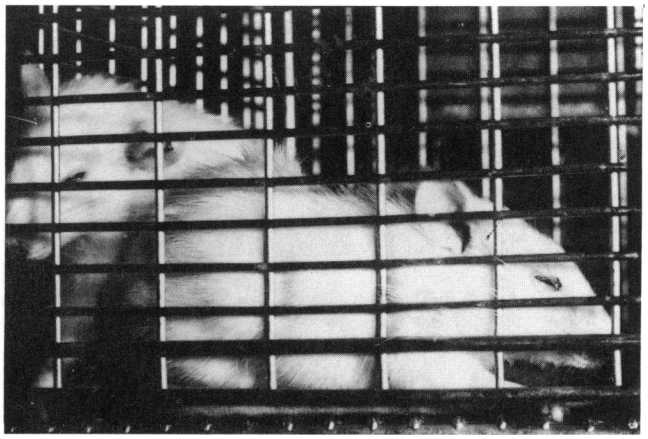

FIGURE 2. Partially closed and wet eyes of rats during methyl isocyanate exposure at 30 ppm.

After the exposure, rats showed dose-related signs of respiratory distress and persistent lacrimation. A reddish-tinged crust was observed around the eyes and nose, and a white crust was apparent around the mouth and under the chin of rats in the 30 ppm group.

Gross Observations

At necropsy, gross examination of eyes with fluorescein sodium under UV light revealed no evidence of epithelial damage to the cornea in animals exposed to 30 ppm of MIC, or in control animals. However, the corneas of those control rats that were lightly scratched manually showed clear evidence of corneal abrasion under UV light.

Histopathologic Observations

Tissues were examined for evidence of treatment related changes such as edema, epithelial erosion, or ulceration of the cornea or adjacent tissues. Microscopic changes related to MIC exposure were not observed in animals examined immediately following, or at other scheduled sacrifice times up to 91 days after the exposures.

Discussion

The symptoms most frequently reported by survivors of the MIC gas leak in Bhopal were burning eyes and throat, and coughing. We observed copious lacrimation and respiratory distress in rats during a 2-hr exposure to MIC at 30 ppm; less severe lacrimation and signs of

FIGURE 3. Blood-tinged frothy discharge from nose (arrows) of rats during methyl isocyanate exposure at 30 ppm.

dyspnea were noted in animals in a 10 ppm exposure. Other signs of eye irritation included rubbing and partial or complete closure of the lids during the exposure. A persistence of lacrimation was suggested by the presence of a reddish crust around the eyes and nose both immediately after exposure and during the first week in high dose rats. This reddish color was likely due to the drying of excess tears which normally contain a porphyrin. A whitish crust was also observed around the mouth and under the chin. This could reflect excess salivation or mucus from the nose during and after exposure.

No evidence of corneal injury was observed in rats following exposure to MIC at doses up to 30 ppm. Exposure to these concentrations resulted in a severe necrotizing effect on the olfactory and respiratory epithelium lining the respiratory tract; no effect was observed in those areas of the nose lined with stratified squamous epithelium (*9*). Stratified squamous epithelium also covers the anterior portion of the cornea, and the apparent lower sensitivity of this cell type to MIC-induced damage may account at least in part for our negative findings. As important or possibly more important are the natural protective mechanisms of the eye that include extensive tearing and eyelid closure which undoubtedly helped minimize direct contact of the irritating vapors to the eye.

In a separate experiment (*10*) in the same laboratory, the cornea and adjoining tissues of the eye of mice exposed to MIC up to 30 ppm have been examined microscopically. No eye lesions related to MIC exposure were detected in mice (*10*).

Although news reports indicate extensive eye injury in people exposed in Bhopal, Andersson et al. (*2*) failed to find any case of blindness or evidence of irreversible eye damage that could be attributed to the gas exposure. Exposure of lens explants *in vitro* to MIC has been shown to result in opacities (*11*), but the relevance of that finding to the human exposure in Bhopal is not clear since to come in contact with the lens, MIC would have to penetrate the cornea.

In summary, rats exposed to 10 and 30 ppm of methyl isocyanate for 2 hr showed eye irritation but no apparent irreversible eye damage. These doses were sufficient to cause severe pulmonary injury and death, but it would appear that the natural protective mechanisms of the rat such as eyelid closure and lacrimation were adequate to prevent significant eye injury.

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