Cardiopulmonary Effects in Awake Rats Four and Six Months after Exposure To Methyl Isocyanate

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Cardiopulmonary function was assessed four and six months after Fischer 344 rats were exposed to 2 hr to 0, 3, or 10 ppm methyl isocyanate (MIC). During assessment, the rats were challenged with 4 and 8% carbon dioxide (CO₂) to stimulate ventilatory drive. Minute ventilation ($V_e$) during CO₂ challenge was increased in MIC-treated rats compared to controls when examined 4 months after exposure to 10 ppm MIC, suggesting a ventilation/perfusion inequality. An increase in maximum expiratory flow and a decrease in expiratory time indicated increased lung recoil in these rats. Evidence of pulmonary hypertension was observed in electrocardiograms (ECGs) and supported by postmortem analysis that showed a positive association between increased ECG abnormalities and increased right ventricular weights in the rats treated with 10 ppm MIC. At 6 months, forced expiratory flow-volume curves indicated persistent airway obstruction; however, no changes in inspiratory or expiratory resistance were evident. Decreased dynamic compliance and changes in two new measures of lung function (volume and time at zero expiratory intrapleural pressure) suggest that MIC-induced lung dysfunction also exhibited elements of a restrictive disease.

Introduction

Breathlessness upon exertion and decreased exercise tolerance were recently reported by Kamat et al. (1), after investigating victims 2 months after the methyl isocyanate (MIC) leak in Bhopal, India. Collectively, their data suggest mild secondary airway obstruction and a moderate restrictive disability with impaired oxygen transport. Clockwise axis deviation in the electrocardiogram (ECG) and radiographic changes, both consistent with the diagnosis of pulmonary hypertension, were also found.

Our pulmonary function data, obtained from anesthetized rats (2), revealed functional evidence of airway obstruction three months after a 2-hr exposure to 10 ppm MIC. The obstructive lesion was characterized by an increased residual volume and end expiratory volume that were apparently the result of gas trapping. As expected, a marked heterogeneity of ventilation was also evident in these animals.

While functional tests performed on anesthetized rats are sensitive for detecting pulmonary injury, they are not appropriate for quantitative assessment of breathlessness during acute periods of exercise or exertion. To evaluate these phenomena, cardiopulmonary function was examined in unanesthetized rats using CO₂ as a ventilatory stimulus that increases both tidal volume ($V_T$) and frequency of breathing ($f$), much like exercise (3). Unanesthetized animals were used because anesthesia blunts nervous system-mediated cardiopulmonary responses such as CO₂-induced stimulation of breathing (4).

Methods

Animals

Male, 6 to 10 week-old Fischer 344 rats (Charles River, Kingston, NY), were exposed to 0, 3, or 10 ppm MIC for 2 hr. After exposure, the animals were group housed in standard plastic cages with filter tops until evaluation. Details of the exposure system and exposure protocol are provided elsewhere (5), as are specifics of animal care, and description of clinical signs and toxicity follow exposures (6). Pulmonary measurements were obtained on two different groups of rats at 4 and 6 months following their exposure on 4/22/85.

Four-Month Study

We routinely measure resistance and compliance in animals to help localize and quantitate the extent of a pulmonary lesion (7). However, because these measurements

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require a surgically implanted intrapleural catheter and because our experience (2) indicated that these rats were still severely affected by 10 ppm MIC at 3 months after exposure, we chose to examine ventilatory responses without the complications of anesthesia or surgical trauma.

**Measurements.** Awake rats, restrained in acrylic holders (8) and placed in head-out plethysmographs mounted on a 0.3-m³ Rochester exposure chamber door (9), were examined during periods of eupneic and CO₂-induced hyperventilation. ECG leads were attached to each rat on the dorsal aspect of each limb prior to securing the plethysmograph (10). A pressure transducer (Validyne Engineering Corp.) attached to each plethysmograph sensed chest expansion and contraction during tidal breathing. This analog signal was continuously displayed on a polygraph and simultaneously digitized by a computer. From the digitized signal, \( V_T, f \), minute ventilation \( V_E \), maximum inspiratory \( V_{\text{Imax}} \) and expiratory \( V_{\text{Emax}} \) flows, and inspiratory \( T_i \) and expiratory \( T_e \) times were computed for each rat.

**Challenge Protocol.** Rats were randomly chosen from the three treatment groups (0, 3, or 10 ppm MIC) and individually tested in groups of four. Measurements were obtained for 7, 12, and 12 rats exposed to 0, 3, and 10 ppm MIC, respectively. Measurements of cardiopulmonary function were obtained five times according to the following protocol (Fig. 1): (a) 30-min filtered air, (b) 15-min 4% CO₂ challenge, (c) 15-min recovery in filtered air, (d) 15-min 8% CO₂ challenge, and (e) 15-min recovery period in filtered air. Carbon dioxide challenge concentrations were produced by metering pressurized CO₂ from a cylinder into the chamber through a computer-actuated mass-flow controller (Sierra Instruments), while the chamber concentration was monitored by a CO₂ analyzer (Beckman Instrument Co.).

Pulmonary measurements were collected during the last 2.5 min of each of the five periods described above. For data collection purposes, the 2.5-min period was divided into five equally spaced, 4-sec epochs, during which time the pulmonary signals were digitized at 250 Hz (9). In conjunction with pulmonary measurements, ECGs were continuously recorded, using an FM cassette recorder (TEAC Corp.), for subsequent analysis following testing (11).

Following pulmonary and ECG data collection, the rats were removed from the plethysmograph, anesthetized with pentobarbital, and killed by exsanguination. The heart and lungs were quickly excised, after which the heart was further dissected into right and left ventricle plus septum, and the remaining combined atria. Wet weights were obtained before the samples were placed in a drying oven and dry weights were obtained 7 days later.

**Six-Month Study**

Preliminary examination of the 4 months post-MIC exposure data revealed that the rats were not excessively compromised during the CO₂ challenge. Therefore, prior to studying their pulmonary response 6 months after MIC exposure, intrapleural catheters were implanted in the rats.

Twenty-five rats were used for this phase of the study. Eight were previously exposed to 3 ppm MIC and 10 were exposed to 10 ppm MIC. The seven control rats were obtained from weight-, sex-, and age-matched Fischer 344 rats housed under similar conditions at a different facility. This substitution was necessary because all paired control rats were utilized in previous experiments.

Rats were handled and challenged with CO₂, and pulmonary measurements collected in a manner similar to the four-month study with the following exceptions: (1) approximately 18-hr prior to testing, rats were anesthetized with pentobarbital, and Silastic intrapleural catheters were aseptically inserted (12); (2) ECG electrodes were not attached; and (3) heart and lung weights were not obtained. Implantation of the intrapleural catheter enabled the following additional measurements to be determined: intrapleural pressure \( P_{\text{pl}} \), dynamic compliance \( C_{\text{dyn}} \), and inspiratory \( R_i \) and expiratory \( R_e \) resistance. Additionally, several new pulmonary function measurements were analyzed to assess the effort-independent portion of the tidal breath. These measurements include the volume \( V_{\text{pl}} \), time \( T_{\text{pl}} \), and flow \( F_{\text{pl}} \) measured when the expiratory \( P_e \) returned to the reference (zero pressure) level for that inspiration.

A forced expiratory maneuver was also performed on these rats after completion of the CO₂ challenge protocol. For this measurement, the rats were anesthetized with pentobarbital, intubated with a transoral tracheal tube, and placed in an acrylic whole-body plethysmograph where the lung was slowly inflated to an airway pressure of 30 cm H₂O. Under computer control, a solenoid valve was opened, exposing the rat's airway to a 40-cm H₂O
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pressure vacuum that caused rapid deflation of the lung (13). During forced expiration, volume and flow signals were digitized by the computer at 1000 Hz, stored, and eventually analyzed to examine airway functional integrity.

Data Analysis

Pulmonary data from the 4- and 6-month studies were analyzed in a similar manner. After completion of the experiment, the five digitized epochs that comprised each of the five sampling periods (Fig. 1) were edited to remove invalid measurements (e.g., movement artifacts, noise, preamplifier saturation). The data from the remaining epochs were averaged so that, per rat, one value for each measurement was obtained for each of the five sampling periods. ECG data were analyzed according to previously described techniques (11).

Profile analysis, a multivariate analysis of variance technique (14), was used to assess whether MIC-treated rats were different in their cardiopulmonary response than control rats. Two separate profile analyses were performed. One analysis examined the profile response of rats at three time points in filtered air (before and after 4 and 8% CO\textsubscript{2} challenge, Fig. 1a,c,e). The second analysis examined the profile response during 0, 4 and 8% CO\textsubscript{2} challenge (Fig. 1a,b,d). Parallel profiles for the three MIC treatment groups, when examined over the three time or CO\textsubscript{2} challenge conditions, were pooled by treatment and tested for difference between MIC treatment groups (0, 3, 10 ppm). If the data were not parallel, a one-way analysis of variance (ANOVA) was used to determine if there was an overall effect due to a single sampling period. This was then followed by a Scheffe’s subtest to examine differences attributable to MIC concentration. ECG data were treated in a similar fashion except only the first filtered air, the 8% CO\textsubscript{2}, and the air recovery samples (Fig. 1a,d,e) were analyzed using the profile analysis technique. Tissue and body weights were compared using ANOVA followed by Bonferroni adjusted t-tests to determine

**Figure 2.** Effect of MIC, 4 months after exposure, on $V_T$ (mL), $f$ (sec), and $V_{E}$ (mL/sec) during 0, 4 and 8% CO\textsubscript{2} challenge. The asterisk (*) indicates significant difference from control.
significant between treatment groups. Significance was accepted if the adjusted probability was < 0.05.

Results

Four-Month Study

The body weights of rats exposed to 10 ppm MIC were significantly depressed (mean ± SD, 354 ± 6 g) compared to both control (389 ± 4 g) and 3-ppm-exposed rats (392 ± 3 g). Profile analysis of the three filtered air samples (Fig. 1a,c,e) indicated that neither time nor residual effects of CO₂ challenge confounded the interpretation of the CO₂ concentration-response profiles (Fig. 1a,b,d) for any variable.

Exposure to increasing concentrations of CO₂ produced significant increases in $V_{T}$, $f$, $V_E$, $V_{i_{max}}$, and $V_{e_{max}}$, and decreases in $T_I$ and $T_e$ in control rats. The response of rats previously exposed to 3 ppm MIC was not different from controls. Rats previously exposed to 10 ppm MIC exhibited a significant 29% increase in $V_E$ and a 22% decrease in $T_I$ during 4% CO₂ exposure, compared to control rats. During exposure to 8% CO₂, $V_{i_{max}}$, $V_T$, and $V_E$ were increased 21, 25, and 26%, respectively, compared to the control group response. For the 10-ppm group, the increase in $V_E$ during CO₂ exposure was primarily due to an increase in $V_T$ (Fig. 2).

Analysis of the electrocardiographic data demonstrated an increased incidence of arrhythmias and other ECG abnormalities in the MIC-exposed animals (Table 1). In most cases, arrhythmias were present during filtered air sampling periods as well as during the CO₂ challenge periods. The most striking finding of these analyses involved the observation of notched R-waves (r'-waves) and slurred S-waves (Fig. 3) indicative of right bundle branch block (15,16). These effects were most pronounced in the 10-ppm rats; however, the 3-ppm-treated rats also exhibited a high degree of general cardiovascular conduction system dysfunction, as evidenced by the large percentage of premature atrial and ventricular contractions and R-R interval variability (Table 1). In contrast, few ECG abnormalities were observed in control rats. During CO₂ challenge, a generalized elevation of the S-T segment and increased electrical instability were observed in all treatment groups. The only abnormal ECG finding was two isolated premature ventricular contractions in one control rat. Profile analysis of the quantitative ECG variables revealed a significant increase in the P-R interval for the 10-ppm group, without a significant change in heart rate or other measured intervals.

When compared to controls, wet and dry lung weight/body weight ratios were significantly increased by 38 and 52%, respectively, in the 10-ppm MIC exposure group. No differences from the control group were found for the measurements in the 3-ppm MIC treatment group, and no differences between the wet/dry weight ratios were seen for either MIC treatment group when compared to controls.

![Control Rat](image1.png)

![10 PPM MIC Treated Rat](image2.png)

**Table 1. Qualitative electrocardiographic irregularities 4 months after MIC exposure.**

| Observation                        | Frequency, %<sup>a</sup> | 3 ppm<sup>b</sup> | 10 ppm<sup>c</sup> |
|------------------------------------|--------------------------|-------------------|-------------------|
| Bundle branch block                | 9                        | 50                |                   |
| Premature atrial contraction       | 36                       | 40                |                   |
| Premature ventricular contraction  | 82                       | 80                |                   |
| Grouped premature ventricular      | 18                       | 10                |                   |
| contraction                        |                          |                   |                   |
| High-peaked P-wave                 | 9                        | 20                |                   |
| Small P-wave                       | 18                       | 10                |                   |
| Negative P-wave                    | 18                       | 10                |                   |
| Multiple P-wave                    | 36                       | 40                |                   |
| Small R-wave                       | 9                        | 40                |                   |
| R-R interval variability           | 18                       | 30                |                   |
| Slurred S-wave                     | 9                        | 20                |                   |
| Large, extended T-wave             | 0                        | 30                |                   |
| Small T-wave                       | 36                       | 20                |                   |

<sup>a</sup> Percentage of rats showing ECG irregularities. Only one control rat showed any ECG abnormality (see text).

<sup>b</sup> $n = 13$.

<sup>c</sup> $n = 11$. 

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**Figure 3.** Top panel, normal ECG waveform ensemble from a control rat during the baseline period (see Fig. 1). Components of the normal rat ECG have been labeled as $P$, $Q$, $R$, $S$, and $T$ waves with subsequent $P$ also shown. Bottom panel, representative sample of an ensemble ECG waveform from a 10-ppm-MIC-treated rat 4 months after exposure. Notice the r'-wave and the slurred S-wave.
The total heart weight/body weight ratio of the 10-ppm group was greater than that of either the 0- or 3-ppm group and could be accounted for by a 33% increase in the weight of the right ventricle (Fig. 4). No other significant differences in heart weights were found. However, a strong positive association existed between increased right ventricle/body weight ratios and qualitative ECG changes. Of the three rats with the most substantial ECG evidence of right ventricular hypertrophy (pronounced r'-waves and slurred S-waves) (Fig. 4), all were from the 10-ppm group, and two of these rats had the highest right ventricular/body weight ratios.

Six-Month Study

The body weights of the 10-ppm-MIC-exposed rats were still significantly reduced (mean ± SD, 379 ± 6) compared to 3-ppm (407 ± 3 g) and to the age and sex-matched controls (415 ± 4 g) group. Unlike the 4-month
study, CO₂ challenge did not reveal any significant changes in ventilatory measurements due to prior exposure to MIC. However, $V_E$ and $V_{t_{max}}$ increased and $T_*$ decreased compared to controls during CO₂ challenge, a pattern that mimicked the results observed in the 10-ppm rats 4 months after MIC exposure.

The additional measurements obtained with the intrapleural catheter did reveal changes associated with exposure to MIC. However, these effects were not enhanced by CO₂ challenge (i.e., the profile responses to CO₂ challenge were parallel). This is in contrast to changes observed in measures of ventilation during the 4-month study where MIC treatment effects were only observed after CO₂ challenge. Dynamic compliance was unaffected during CO₂ challenge, but was decreased in both the 3-ppm and 10-ppm exposure groups, with the effect on the 10-ppm group being significant (Fig. 5). Pleural pressure increased with increasing CO₂ concentration and tended to be slightly higher in 10-ppm-MIC-treated rats, however, MIC treatment did not change $R_i$ or $R_e$.

Two of three new pulmonary function measurements proved to be sensitive to MIC treatment and CO₂ challenge. While both volume and flow at zero $P_{pl}$ were increased by increasing CO₂ concentrations, only $V_{zp}$ was
significantly decreased by 3- and 10-ppm MIC treatment. During the first filtered air sampling period, \( V_{\text{EP}} \) was 19% less than control for the 3-ppm group and 35% less for the 10-ppm group (Fig. 6). Similarly, \( T_{\text{EP}} \) was decreased by 8% at 3 ppm, and by 34% at 10 ppm; however, only the effects at 10 ppm were significant (Fig. 6).

 Forced expiratory measurements were also altered with MIC lung injury (Fig. 7). Peak flow and the forced expiratory flow at 50% of vital capacity were significantly reduced for both the 3- and 10-ppm MIC treatment groups. Expiratory flows at 25% and 10% of the forced vital capacity were also significantly reduced for the 10-ppm group.

**Discussion**

MIC has been shown to be a potent sensory as well as pulmonary irritant in mice and guinea pigs during exposure (17,18). Our studies with anesthetized rats (2) indicate persistent airway obstruction and restriction through 3 months following a single 2-hr exposure to 10 ppm MIC. This report suggests that, as long as 4 and 6 months after MIC exposure, functionally restrictive and obstructive lesions persist, and that pulmonary hypertension may be a secondary consequence of this chronic lung injury. Evidence for these conclusions follows.

An increase in \( V_e \) was observed in the rats 4 months after exposure to 10 ppm MIC, but only during \( CO_2 \) exposure. The increase in ventilation may be due to an increased responsiveness to \( CO_2 \), or may indicate inadequate oxygen entering the blood. These data complement preliminary reports from Bhopal that describe breathlessness upon exertion and decreases in \( P_{AO_2} \) during rest and after exercise (1). However, our data at 6 months after exposure indicate no difference between control \( V_e \) and the \( V_e \) of rats previously exposed to 10 ppm MIC. It is possible that the surgical procedure (which was only performed at 6 months) blunted the effect of MIC (9).

Although the increase in \( V_e \) is not consistent with the rapid shallow pattern seen in pure restrictive-type disease, the increase in \( V_{\text{max}} \) and decrease in \( T_e \) observed at 4 months after exposure suggest increased lung recoil (i.e., restrictive pattern). Further evidence of a functionally restrictive lung was indicated by the decreased \( C_{\text{dyn}} \) (a measure of lung elasticity) without a change in \( R_1 \) or \( R_2 \) (measures of upper airway obstruction) in the rats studied 6 months after exposure. The measurements of \( V_{\text{EP}} \) and \( T_{\text{EP}} \) indicate less volume remaining in the lung with proportionally less time to empty the remaining volume. Interpretation of such data would suggest that the lung recoils faster, despite the return of the intrapleural pressure to reference level (i.e., ready to begin a new breath). These data are also consistent with a functionally restricted lung during tidal breathing.

In contrast, the data from the forced expiratory maneuver in the same rats, after they were anesthetized and intubated, revealed continued functional airway obstruction as was observed by Stevens et al. (2). Reductions in the peak flow and expiratory flow at 50% forced vital capacity in both the 3-ppm and the 10-ppm group indicate a continued involvement of the large airways, which were the site of initial damage (19). In addition, the significant decreases in forced expiratory flow at 25 and 10% forced vital capacity reveal a more pervasive involvement, that

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**Figure 7.** Effect of MIC, 6 months postexposure, during a forced expiratory maneuver. Data are expressed as maximum flow versus percent forced vital capacity. Bars indicate SE from flow axis. Bars at peak flow also indicate SE about both volume and flow axes. The asterisk (*) indicates significant difference from control.
includes damage to the small airways of the lungs of rats previously exposed to 10 ppm MIC (20). This obstructive component of lung damage may have been observed during our measurements of tidal breathing in awake rats because of the limited volume range examined.

Since previous data (2) did not show effects following 3-ppm MIC exposure, it is possible that surgical trauma or the substitute control group used in the 6-month study in some way affected the results. Introduction of the pleural catheter and residual effects 18 to 24 hr after pentobarbital anesthesia may have further compromised an already damaged lung. With regard to the suitability of the control group, it should be noted that mature, 9-month-old, Fischer 344 rats are fairly uniform in size and pulmonary responses (21–22). Additionally, the 3-ppm group was statistically different from the control group in only 3 of 18 measured variables, with those three variables affected in a concentration-related manner.

Evidence of pulmonary hypertension included the increase in right ventricular weight/body weight ratio, as well as r’-waves and slurred S-wave in the 10-ppm MIC treatment group. The positive correlation between right ventricular size and the observed ECG abnormalities further strengthens these conclusions. Such ECG abnormalities indicate right bundle branch block, which frequently develops following prolonged strain on the right ventricle or in conjunction with right ventricular hypertension (RVH). Both right ventricular strain and RVH are natural consequences of pulmonary hypertension (15,16).

Pulmonary hypertension probably occurred as a result of injury to the peribronchiolar region of the lung (19). Obstructed airways or restrictive lesions closing airways cause hypoxia, which leads to pulmonary vascular constriction and increased pulmonary resistance, resulting in pulmonary hypertension (24). However, pulmonary hypertension may have also resulted from initial damage to the vasculature surrounding the peribronchiolar region during MIC exposure.

In summary, Fischer 344 rats showed changes in ventilation and an increase in lung recoil 4 months after exposure to 10 ppm MIC. These same rats showed an increased incidence of ECG abnormalities and RVH. Right ventricular hypertrophy is almost always secondary to pulmonary hypertension, which in the present study is likely to be secondary to lung damage. This lung damage, still present 6 months after exposure, had obstructive components, as indicated by the changes observed in the forced expiratory flow volume curves, and restrictive components, as shown by the decrease in $C_{dys}$, $V_{pl}$, and $T_{pl}$. These conclusions agree well with recent data obtained from humans exposed to MIC in Bhopal, India (1).

The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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