Environmental Health Perspectives
Vol. 72, pp. 149-152, 1987

Methyl Isocyanate: Reproductive and Developmental Toxicology Studies in Swiss Mice

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Studies were conducted in Swiss (CD-1) mice to evaluate the potential of inhaled vapors of methyl isocyanate (MIC) to affect reproduction and development. Inhaled MIC at concentrations of 0, 1, or 3 ppm, 6 hr per day during days 14 through 17 of gestation caused a significant increase in the number of dead fetuses at birth and caused a significant decrease in neonatal survival during lactation. In contrast, exposure of male and female mice to 1 or 3 ppm given 6 hr per day for 4 consecutive days had no effect on reproduction during mating trials conducted 1, 8, and 17 weeks after the exposure period. Similarly, there was no evidence of a dominant lethal effect in exposed male mice.

Introduction

Methyl isocyanate (MIC) is a chemical characterized as highly reactive, toxic, volatile, and flammable. The accidental exposure of people in Bhopal, India, to vapors of MIC on December 3, 1984, resulted in the deaths of more than 2,000 people. This accident prompted the conduct of a series of toxicological studies in laboratory animals, including the one reported here. Previously published toxicological data on this chemical are scarce. Kimmerle and Eben (*1*) studied the acute toxicological properties of inhaled MIC and reported this chemical to be extremely irritating to mucous membranes. Pozzani and Kinkead (*2*) reported on the toxicity of inhaled MIC, including its potential to cause severe bronchial spasms and asthmalike breathing in rats and mice. These studies identified severe burns following dermal contact and the hazards posed with contact to the eye and other mucous membranes. Like other isocyanates, MIC caused sensitization in animals and produced cross-sensitization to other isocyanates. While the respiratory tract was considered to be the primary target organ for the toxic effects of methyl isocyanate, the studies reported here were conducted to evaluate the effects of sublethal concentrations of inhaled methyl isocyanate on reproduction and developmental toxicity in mice exposed during late gestation or prior to mating.

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Materials and Methods

MIC was provided by the Agricultural Products Co., Inc. of the Union Carbide Corporation (Research Triangle Park, NC). Purity of greater than 99% MIC was confirmed by gas chromatography with flame ionization detection. Sexually mature Swiss (CD-1) male and female mice, obtained from Charles River Laboratories, Inc. (Portage, MI), were used for these studies. Except for time-dated pregnant mice, all other mice were acclimated for a minimum of 10 days prior to random assignment to treatment groups within studies. Mice were randomized by weight to minimize potential differences between groups. All mice were identified by ear tag before initiation of studies and were observed daily throughout the studies for signs of toxicity. Mice were housed in sanitized stainless-steel, wire-mesh exposure cages during exposure periods. After exposure, animals were held in the exposure chambers for an additional 45 min to allow purging of the chamber environment, after which they were transferred to plastic animal-holding cages for feeding and watering. Filter tops were used on animal cages during overnight holding. Commercial laboratory chow (NIH-31, Zeigler Brothers, Inc., Gardner, PA) and deionized water were available free choice during nonexposure intervals. Ambient temperature ranged from 20 to 27°C, and relative humidity ranged from 31 to 64%. All animals were on a 12-hr light:dark cycle.

Experimental Design

Perinatal Toxicity Study

Groups of 39 to 44 female Swiss (CD-1) mice were exposed on gestation days 14 through 17, 6 hr per day, to concentrations of 0, 1, or 3 ppm MIC. These mice were permitted to deliver their offspring for evaluation of neonatal survival and development. Adult female mice were weighed on days 14 and 18 of gestation and on day 21 of lactation. The offspring were weighed, counted, and sexed on the first day after delivery and were weighed and counted again on days 4, 7, 14, and 21. Litters were culled to eight pups by random selection on day 4, maintaining equal numbers of each sex when possible. The following data were also collected: number of days from mating to parturition, number of live and dead offspring at delivery, mean pup weights, and the number of pregnant females with live pups. Pups were observed for their demeanor and any evidence of external structural malformations.

Mating Trials

Thirty male and female Swiss (CD-1) mice per dose group were used for mating trials following 4 consecutive days of exposure, 6 hr per day, at concentrations of 0, 1, or 3 ppm MIC. Mating trials were conducted during weeks 1, 8, and 17 following exposure. Treated males and treated females from the same exposure group were permitted to cohabit for 10 consecutive days. During cohabitation, the female mice were checked daily for presence of vaginal plugs as evidence of mating. Cohabitation continued for 10 days or until evidence of mating occurred. The day on which a vaginal plug was found was considered day 0 of pregnancy.

The females were permitted to deliver their litters, and the pups were observed until 21 days of age. The observations on the neonates were the same as those described above for the perinatal toxicity studies.

Table 1. Summary of methyl isocyanate inhalation exposure chamber concentrations determined during a repeated dose (6 hr/day × 4 days) study with female mice.[a]

Exposure date	Concentration of MIC, ppm — Exposure group, ppm 1	3
4–11–85	1.07 ± 0.05 (66)[b] [0.96, 1.15]	2.95 ± 0.34 (67) [1.38, 3.38]
4–12–85	0.92 ± 0.08 (64) [0.79, 1.16]	2.97 ± 0.54 (64) [2.48, 4.43]
4–13–85	1.01 ± 0.05 (65) [0.84, 1.09]	3.06 ± 0.31 (66) [2.52, 3.82]
4–14–85	1.04 ± 0.07 (66) [0.95, 1.23]	3.01 ± 0.18 (66) [2.72, 3.60]
Total average concentration	1.01 ± 0.07 (269) [0.79, 1.23]	3.00 ± 0.18 (263) [1.38, 4.43]

[a] Mice were exposed 6 hr/day for 4 consecutive days coinciding with gestation days 14 through 17.
[b] Mean ± SD; numbers in parentheses represent number of data points; numbers in brackets represent the minimum and maximum concentration during exposure period.

Dominant Lethal Study

Thirty male Swiss (CD-1) mice per dose group were mated with untreated females for 8 weeks (different females each week) following the last of 4 consecutive days of exposure, 6 hr per day, to 0, 1, or 3 ppm MIC. Male mice were cohabited with female mice on a one-to-one basis. The animals were observed daily for signs of toxicity and twice daily for any moribund or dead mice. The female mice were replaced with new untreated female mice of the same age at 7-day intervals. Male mice were weighed on the day before the first exposure, the first day after the fourth exposure, and at weekly intervals when the female mice were replaced. Following removal from the exposed males, the female mice were housed separately and were sacrificed 11 days after removal from the males. At the time of sacrifice, the uterine contents were examined for pregnancy and the number of live and dead implants to determine the percent of resorptions relative to the number of implantation sites.

Inhalation Exposure

All exposures of mice were conducted in the inhalation facility at the National Institute of Environmental Health Sciences, Research Triangle Park, NC. These facilities are certified by the American Association for the Accreditation of Laboratory Animal Care. The specific details for the generation of MIC vapors and the analysis of their concentration in the exposure chambers, as well as measurements for the presence of MIC in room air and chamber effluent, are reported in a publication by Adkins et al. (*3*).

The concentrations of MIC achieved in the chambers during the exposures are summarized in Table 1. Because of the extreme closeness of the analyzed concentrations to the intended nominal concentrations, reference throughout this paper is made only to the nominal concentrations. All MIC concentrations were

Table 2. Fetal and neonatal deaths among litters of MIC-exposed female CD-1 mice.[a]

Time of deaths	% Dead — Exposure group, ppm 0	1	3
At birth	0.4 (2/461)[b]	3.3 (9/373)[e]	6.4 (22/341)[e]
Days 0–4	2.0 (9/459)[c]	0.8 (3/364)	11.3 (36/319)[e]
Days 5–21	0.3 (1/340)[d]	0.4 (1/271)	2.9 (7/239)[e]

[a] Mice were exposed 6 hr daily on gestation days 14 through 17.
[b] % (no. dead/no. live or dead).
[c] % (no. dead/no. live on day 0).
[d] % (no. dead/no. live on day 4 after culling).
[e] Significantly different from control by Fisher's exact probability test, $p < 0.05$.

maintained within approximately 10% of the target concentrations throughout the exposure period.

Statistical Evaluation

Body weight data were analyzed by an analysis of variance and Dunnett's test (*4*). Fertility indices were analyzed by Fisher's exact probability test (*5*). The resorption data were analyzed using the Wilcoxon test as modified by Haseman and Hoel (*6*). A level of significance chosen in all cases was $p < 0.05$.

Results

Perinatal Toxicity Study

Exposure of pregnant Swiss (CD-1) mice on days 14 through 17 of gestation to vapors of MIC had no effect on maternal survival, body weight, demeanor, or the length of gestation. All pregnant females delivered litters with one or more live pups. There was, however, a significant adverse effect on the number of fetal deaths observed at birth and on neonatal survival during lactation. As shown in Table 2, there was a significant increase, compared to controls, in the number of dead fetuses observed at birth at both 1 and 3 ppm MIC. This increase in mortality of the fetuses continued, as there was increased mortality among the neonates throughout lactation. The increased mortality in neonates was somewhat higher on days 1 through 4 than it was during the remainder of the lactation period. The weights of the neonates at birth and during lactation were no different between the MIC groups and the controls. There was also no effect on demeanor or external abnormalities. The increase in neonatal mortality was observed as a significant decrease in live litter size (Table 3), which was particularly evident prior to the time of standardization of litter size to eight pups at 4 days of age.

Mating Trials

No significant adverse effects were observed in mating trials conducted on male and female mice exposed to MIC vapors. There was no effect on body weight, demeanor, fertility, or litter size. As shown in Table 4, a slight decrease in fertility was observed in the 3 ppm group in the litters which resulted from the second mating, during the week 8 after exposure to MIC. In view of this questionable effect on fertility, all pairs of animals were mated for a third time during week 17 after exposure. During this mating, there was no adverse effect on fertility at either exposure concentration of MIC, but fertility in all groups, including controls, was slightly lower than during the two previous mating periods.

The litter size at birth was not affected by parental exposure to MIC (Table 4). Likewise, survival of neonates throughout lactation was not compromised in any of the three sets of matings. Representative members of the F_{1a} litters which were selected for a subsequent mating trial at maturity (75 days of age) showed no adverse effects on fertility or litter size at birth of the F_{2a} generation.

Dominant Lethal Study

No evidence of a dominant lethal effect was observed in male mice exposed to MIC (Table 5). The fertility of these male mice mated with unexposed females during 8 weekly intervals following exposure was comparable to control values. There was no effect on the incidence or distribution of resorptions in the pregnant females mated to the treated males.

Discussion

These studies evaluated the potential effects of inhaled methyl isocyanate at sublethal concentrations on reproductive and developmental processes in mice. The concentrations of MIC used in these studies were selected on the basis of concurrent toxicology studies conducted in our laboratories. In parallel studies in B6C3F1 mice and F344 rats (NTP unpublished results), significant pathologic changes, particularly of the respiratory tract, were observed under the same exposure conditions used for the reproductive studies reported here.

Table 3. Litter size among MIC-exposed female CD-1 mice.[a]

	Average number of pups/litter		
	Exposure group, ppm		
Day	0	1	3
0 (birth)	10.4 ± 2.0[b]	8.7 ± 4.3	8.0 ± 3.3[c]
1	10.3 ± 2.0	8.7 ± 4.3	7.8 ± 3.3[c]
4	10.2 ± 2.1	8.6 ± 4.4	7.1 ± 3.9[c]
4 (culled)	7.7 ± 0.8	6.4 ± 2.9	6.0 ± 2.9
7	7.7 ± 0.8	6.4 ± 2.9	5.8 ± 3.0
21	7.7 ± 0.8	6.4 ± 2.9	5.8 ± 3.0

[a] Mice were exposed 6 hr daily on gestation days 14 through 17.
[b] Mean ± SD of live and dead fetuses combined.
[c] Significantly different from control by a modified Wilcoxon test, $p < 0.05$.

Table 4. Fertility of MIC-exposed male and female CD-1 mice and live litter size at birth.[a,b]

		Exposure group, ppm		
	Litter	0	1	3
No. of pairs		30	30	30
% pregnant (no.)	F_{1a}	90 (27)	97 (29)	97 (29)
	F_{1b}	93 (28)	97 (29)	83 (25)
	F_{1c}	83 (25)	83 (25)	80 (24)
Live litter size at birth[c]	F_{1a}	11.6 ± 1.6	10.9 ± 1.8	11.4 ± 1.3
	F_{1b}	12.5 ± 2.3	12.1 ± 2.1	12.2 ± 2.1
	F_{1c}	10.8 ± 3.7	11.3 ± 3.4	12.4 ± 2.6

[a] Male and female mice were exposed 6 hr daily for 4 consecutive days; F_{1a}, F_{1b}, F_{1c} litters resulted from matings during weeks 1, 8, and 17, respectively, after exposure.
[b] Values from the treated groups did not differ significantly from the control groups, $p < 0.05$.
[c] Mean ± SD.

In addition, exposure of B6C3F1 mice to 6 ppm MIC 6 hr daily for 4 days killed 19 of 20 males and 11 of 20 females within 14 days. There were no deaths among B6C3F1 mice at concentrations of 3 or 1 ppm and no deaths of the exposed male or female mice in the reproductive studies reported here. Thus, the concentrations of MIC used in these reproductive studies were sufficiently high to cause toxic effects in the known target organs for this chemical, and concentrations slightly higher than those used in these studies caused significant lethality in mice.

The only adverse reproductive effect observed in these studies was a significant increase in the incidence of fetal deaths among litters of female mice exposed during late pregnancy, and a subsequent and related increase in death of neonates during lactation among litters of mice exposed during pregnancy. The fetal deaths observed in the litters of exposed pregnant females were not accounted for by the complete loss of a few litters, but were distributed among many litters of all sizes. During lactation, the deaths of pups of MIC-exposed mothers tended to be distributed among many litters, but there were several litters in which all pups died. Whether the fetal and neonatal deaths were a direct or indirect effect of MIC exposure is uncertain, but it is possible that the deaths were secondary to acute toxic effects from inhalation of MIC.

In contrast to the effect when there was exposure during pregnancy, there was no effect on fertility or on fetal or neonatal survival when males or females were exposed to MIC prior to mating. This suggests that exposure of a conceptus *in utero* results in more toxicity than exposure of the gonadal cells prior to mating.

The lack of a dominant lethal effect in these exposed mice is consistent with the minimal evidence for mutagenic or genotoxic effects in other *in vivo* tests as part of these studies to characterize the toxicity of inhaled MIC. Whether the lack of effect in these studies is related to the nongenotoxicity of MIC or related to the failure of MIC to reach critical target cells is unknown. However, evidence of genotoxicity was observed in *in vitro* tests by Shelby et al. (*7*).

There are some critical differences between the exposure of animals to MIC in these studies and the accident involving MIC in Bhopal, India, in 1984. Some of the people in Bhopal were undoubtedly exposed to much higher concentrations of MIC than were used in these studies. While there were no deaths among the adult mice exposed to MIC in these studies, the slope of the dose-response curve for MIC-induced toxicity is quite steep. Exposures of mice to MIC at concentrations slightly higher than 3 ppm were fatal. Thus, the 3 ppm for 6 hr selected for these studies closely approaches the lethal level in mice.

Another difference between the Bhopal exposure and those used in our studies is that the mice in our studies were exposed to pure MIC vapors; the people in Bhopal were exposed to MIC along with other reaction mixtures from the explosion. The different reaction products from the Bhopal accident could produce a profile of toxicity different from that associated with MIC itself.

In summary, exposure of pregnant Swiss (CD-1) mice to concentrations of 1 or 3 ppm MIC on days 14 through 17 of gestation caused a significant increase in the number of dead fetuses at birth as well as a significant decrease in neonatal survival. The same concentrations of MIC had no effect in mating trials conducted on exposed male and female mice. Similarly, no dominant lethal effect was observed in exposed male mice mated to untreated female mice.

Table 5. Dominant lethal test in MIC-exposed male CD-1 mice.[a]

	Week	Exposure group, ppm 0	1	3
No. of males		30	30	30
% pregnant females (no.)	1	93 (28)	93 (28)	97 (29)
	2	93 (28)	93 (28)	83 (28)
	3	97 (29)	83 (25)	97 (29)
	4	100 (30)	93 (28)	100 (30)
	5	100 (30)	97 (29)	100 (30)
	6	93 (28)	93 (28)	100 (30)
	7	93 (28)	87 (26)	100 (30)
	8	97 (29)	93 (28)	100 (30)
% Resorptions, mean ± SD	1	5.6 ± 8.3	4.9 ± 6.2	4.3 ± 6.4
	2	5.3 ± 8.8	4.7 ± 6.5	4.1 ± 5.4
	3	6.8 ± 11.5	5.2 ± 7.0	5.5 ± 7.0
	4	2.6 ± 4.9	2.3 ± 4.3	4.3 ± 6.9
	5	5.5 ± 6.3	6.7 ± 18.6	4.5 ± 5.7
	6	5.2 ± 8.2	5.3 ± 10.6	6.1 ± 9.4
	7	6.6 ± 6.7	3.7 ± 5.2	3.5 ± 6.2
	8	7.7 ± 8.3	6.2 ± 6.8	5.1 ± 7.9

[a] Values from the treated groups did not differ significantly from the control groups, $p < 0.05$.

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