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Epidemiological and Experimental Studies on the Effects of Methyl Isocyanate on the Course of Pregnancy*

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Although press reports indicate that the leakage of methyl isocyanate (MIC) on December 3, 1984, in Bhopal has led to an increase in spontaneous abortions, stillbirths, infant mortality, and fetal abnormalities, no clinical or experimental studies on the reproductive toxicity of MIC were reported in scientific journals for several months after the accident. We therefore conducted, 9 months after the accident, a preliminary survey of 3270 families in Bhopal and experimental studies on the effects of MIC in pregnant mice. It was found that 43% of pregnancies in women residing near the Union Carbide pesticide plant did not result in the birth of a live child. Likewise, exposure of mice to relatively low concentrations of MIC (9 and 15 ppm) for 3 hr caused complete resorption in more than 75% of animals. A decrease in fetal and placental weights was observed at 2 to 15 ppm MIC. In general, the experimental findings in mice corroborate the epidemiological data from Bhopal. The mechanism of the fetal toxicity of MIC remains to be established.

Introduction

Both the magnitude of the Bhopal accident of December 3, 1984, and the paucity of data on methyl isocyanate (MIC) toxicity (*1*) led to apprehensions of long-term pulmonary, nonpulmonary, obstetric, and gynecologic complications in the affected population (*2*). Approximately 6 weeks after the accident, some evidence of the adverse effects in pregnant women had already become apparent (*2*). In subsequent months, reports of an increase in stillbirths, spontaneous abortions, fetal abnormalities, and infant mortality appeared in survey findings (*3,4*), press interviews (*5*), and conference proceedings (*6*). However, in the absence of the publication of the findings of the Indian Council of Medical Research (ICMR), which is in charge of the follow-up of the Bhopal victims, and because of numerous legal, economic, and political considerations, the nature and the magnitude of the effects of MIC exposure on the course of pregnancy remains uncertain. We therefore conducted both a preliminary survey of the areas adjacent to the Union Carbide India Limited pesticide plant 9 months after the accident and an experimental study on the effects of MIC in pregnant mice at the Graduate School of Public Health, University of Pittsburgh (*7*).

*Experimental studies on mice were conducted at the Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261.

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Methods

Epidemiological Studies

The survey was taken during the first 2 weeks of September 1985, nearly 9 months after the accident. A total of 3270 families who were residing adjacent to the Union Carbide pesticide plant and were exposed to MIC vapor during the accident were surveyed. Retrospective history of the same families for the 2 years prior to the accident served as the control. This approach was used to minimize variables such as socioeconomic factors and response of the subjects to the questionnaire. The actual survey was conducted by high school graduates familiar with the area. The students used a printed list of questions written in Hindi, the local language. These students were instructed on the purpose of the survey, urged to explain to each family that the survey had nothing to do with any type of compensation, and were asked to accurately record answers as stated by the subjects. Subjects who were pregnant at the time of the accident were asked if the pregnancy was successful or unsuccessful; as a partial check on the information, subjects were also asked if an abortion had occurred. A discrepancy of approximately 2% was noted between the two answers; 379 of the 865 women said they were pregnant at the time of the accident but did not deliver a live baby, and 388 said an abortion occurred. Data from the survey were then entered for analysis into a microcomputer by a Hindi-speaking person other than the author.

Experimental Studies

Pregnant Swiss-Webster mice weighing approximately 25 g were purchased from Hilltop Laboratories (Scottdale, PA). The presence of a vaginal plug was taken to indicate day 0 of gestation. Animals had free access to tap water and mouse chow and were maintained on a 12-hr light/dark cycle. All animals were exposed to methyl isocyanate (MIC) vapor only once for 3 hr on day 8 of gestation. The exposure method has been described elsewhere (*7,8*). Mice were sacrificed on day 18 of gestation. Resorptions, live and dead fetuses, and any external, visceral (*9*), or skeletal (*10*) abnormalities were recorded according to standard procedures. In a limited number of experiments, the effect of IP injection of liquid methyl isocyanate into 8-day pregnant mice and 13-day pregnant Sprague-Dawley rats was also studied. MIC was injected by means of a gas-tight 10 μL Hamilton syringe.

Serum progesterone and corticosterone were quantitated by radioimmunoassay (*11*). Means were compared using Student's *t*-test or an analysis of variance.

Results

Clinical Findings

Of the 3270 families surveyed, 865 women reported that they were pregnant at the time of the accident; 43.8% of these pregnancies did not lead to the birth of a live baby. Of the 486 live births, 14.2% of infants died within 30 days as compared with an infant death rate of 2.6 to 3% within 30 days after birth in the 2 years preceding the accident. The rate of loss of pregnancy appeared to be higher in women who were in their first trimester during the accident than in women who were in their second or third trimester of pregnancy at the time of the accident (Fig. 1).

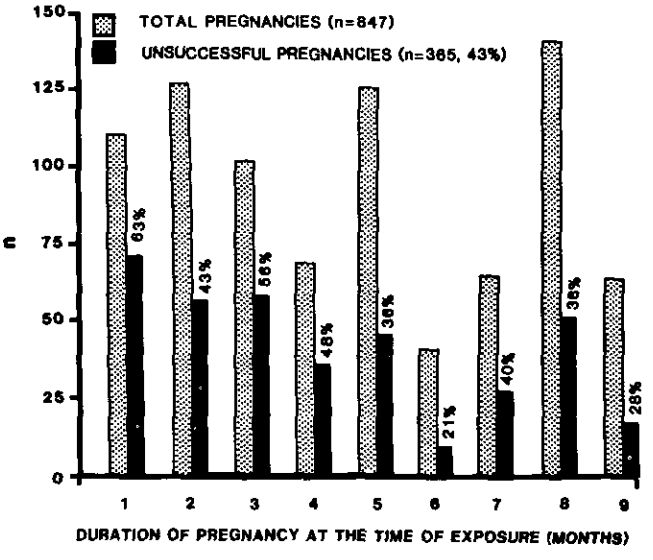

FIGURE 1. Outcome of pregnancies in women exposed to methyl isocyanate gas during the December 3, 1984, accident in Bhopal. Data are based on a survey conducted during September 1985. Unsuccessful pregnancies include all cases (stillbirths, spontaneous abortions, and possibly intentional abortions) in which the woman said she was pregnant and did not give birth to a live baby. The duration of pregnancy at the time of the accident is as stated by the subjects.

Experimental Data

Exposure of mice to MIC caused a reduction in body weight gain. The maximum decrease in body weight occurred 48 hr after the expsoure to MIC, after which body weight gain equaled that of controls. However, the body weights of exposed mice never caught up with controls, so the body weight curve is parallel to and on the right of the control body weight curve. Exposure to air exerted no significant effect on body weight (Fig. 2).

The main effect of a single exposure of pregnant mice to MIC for 3 hr was a concentration-dependent increase in embryo loss: at 9 and 15 ppm MIC more than 75% of animals lost all their fetuses. If the animals retained pregnancy, the number of resorptions, as well as the number of dead fetuses, was too small to influence the litter size. At all concentrations, MIC caused a decrease in fetal and placental weights. Exposure to air exerted no significant effect on the outcome of pregnancy (Table 1).

There was no evidence of external malformation, ex-

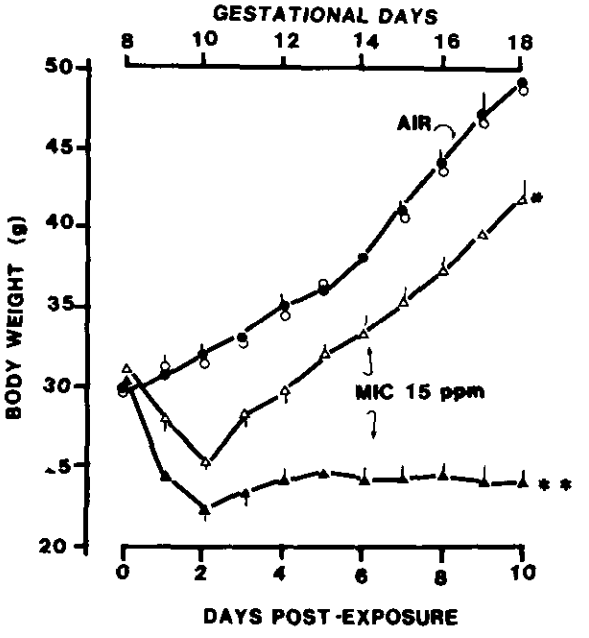

FIGURE 2. Effect of methyl isocyanate (MIC) vapor on the body weight of pregnant animals. Exposure to air or MIC was conducted on day 8 of gestation for 3 hr. Not exposed (o—o, $N = 10$); exposed to air (●—●, $N = 24$); exposed to MIC 15 ppm and animals retained pregnancy (△—△, $N = 4$); exposed to MIC 15 ppm but animals did not retain pregnancy (▲—▲, $N = 12$). *Significantly different from the corresponding body weights in unexposed and air-exposed animals on all days except that on the pre-exposure day (day 0), * $p < 0.05$; ** $p < 0.01$.

Table 1. Maternal and fetal toxicity of methyl isocyanate (MIC) vapor in mice.[a]

Treatment	Animals, N	Maternal mortality, N	Surviving dams losing all embryos, %	Fetal body weight, g	Placental weight, g
None	10	0	0	1.5 ± 0.04	97 ± 3
Air	24	0	0	1.5 ± 0.02	95 ± 2
MIC 2 ppm	11	0	9	1.4 ± 0.02*	88 ± 3*
MIC 6 ppm	12	0	8	1.4 ± 0.03*	85 ± 2*
MIC 9 ppm	12	2	80	1.1 ± 0.02*	80 ± 1*
MIC 15 ppm	18	2	75	1.3 ± 0.04*	74 ± 3*

[a] Animals were exposed for 3 hr to air or MIC on day 8 of gestation and sacrificed on day 18 of gestation.
* Significantly different from the values for the unexposed or air-exposed animals ($p < 0.05$).

cept the presence of meningocele in one fetus of a 9 ppm MIC-treated mouse. However, in 24 randomly selected fetuses from MIC-exposed animals, there was a thinning of the myocardium in two, diaphragmatic hernia in two, hydronephrosis in one, and cleft palate in one. In 76 randomly selected control fetuses, there was hydronephrosis in one and cleft palate in one. MIC also caused approximately a 20% reduction in the lengths of the mandible and bones of the extremities (Table 2).

MIC caused a significant increase in serum corticosterone in nonpregnant mice. This increase was maximal 1 day after the exposure and was followed by a decline and then a second phase of increase (Table 3). Serum corticosterone levels (702 ng/mL at 9 ppm MIC; 557 ± 41 ng/mL at 6 ppm MIC) of MIC-exposed pregnant mice were not significantly higher than the values in pregnant control mice (560 ± 30 ng/mL (*7*). If exposure to MIC led to resorption of all fetuses, serum corticosterone levels (198 ± 93 ng/mL) significantly decreased relative to values in animals which retained the pregnancy. Likewise, when the exposure to 9 ppm MIC caused resorption of all fetuses, serum progesterone levels were 1.7 ± 0.4 ng/mL and significantly lower than the values in pregnant control animals (3.7 ± 0.7 ng/mL). It was not clear if the fetal loss preceded or followed changes in hormonal levels.

In order to find out if pulmonary involvement was essential for the fetal toxicity, MIC was injected IP into a group of pregnant mice. The minimum dose that could be injected (80 mg/kg, 2 μL/mouse) was lethal to all animals ($N = 5$) in less than 16 hr. These animals exhibited signs of sytemic toxicity such as a decrease in spontaneous activity, difficulty in breathing, central nervous system excitation such as that caused by cholinesterase inhibitors, paralysis, and death. It was, however, possible to inject as little as 10 mg/kg of MIC into rats and achieve an increase in resorptions, but this caused only a slight decrease in fetal and placental weights (Table 4).

Discussion

The epidemiological and experimental data reported here clearly indicate that the exposure to methyl isocyanate can adversely affect the course of pregnancy. These studies confirm the reported increase in stillbirths and spontaneous abortions in Bhopal in the post-accident period (*2,5*). However, a more controlled and systematic epidemiological study than the one reported in this paper and a longer follow-up are needed to more precisely establish the magnitude and nature of the adverse effects of the MIC leakage on the course of pregnancy in Bhopal.

Although the survey relied entirely on the information provided by the victim or a senior member of the family and does not involve any objective assessment, the data are probably as reliable as they can be. For example, there is only a 2% discrepancy in relation to the reporting of a pregnancy terminated by abortion, as compared with the reporting of a pregnancy not culminating in the birth of a live child. The higher incidence of pregnancy loss in women who were in their first

Table 2. Effect of methyl isocyanate (MIC) vapor on the skeleton lengths of mouse fetuses on day 18 of gestation.

Parameters	Exposed for 3 hr on day 8 of gestation to: Air	MIC, 6 ppm	MIC, 9 ppm	MIC, 15 ppm
Litters examined, N	22	11	2	4
Fetuses examined, N	46	22	7	14
Mandible, mm	5.2 ± 0.08[a]	5 ± 0.13	4.7 ± 0.07*	4.8 ± 0.1*
Humerus, mm	2.9 ± 0.03	2.9 ± 0.02	2.4 ± 0.03*	2.6 ± 0.1*
Radius, mm	3.2 ± 0.05	3.2 ± 0.05	2.6 ± 0.06*	2.7 ± 0.08*
Ulna, mm	2.5 ± 0.04	2.5 ± 0.07	2.0 ± 0.02*	2.1 ± 0.06*
Femur, mm	2.3 ± 0.04	2.3 ± 0.07	1.9 ± 0.02*	2.1 ± 0.07*
Tibia, mm	2.8 ± 0.06	2.9 ± 0.06	2.1 ± 0.02*	2.2 ± 0.06*
Fibula, mm	2.6 ± 0.04	2.5 ± 0.07	2.1 ± 0.01*	2.2 ± 0.07*

[a] Values are mean ± SE.
* Significantly different from the corresponding control values ($p < 0.05$).

Table 3. Effect of methyl isocyanate (MIC) vapor on serum corticosterone of nonpregnant mice.

	Corticosterone (ng/mL) treatment[a,b]	
Days of postexposure	Air	MIC 9 ppm
1	21 ± 6	139 ± 18*
7	—[c]	29 ± 14**
12	19 ± 2	88 ± 22*

[a] Data are means ± SE from 5–14 animals.
[b] Exposed for 3 hr.
[c] Not analyzed.
* Significantly ($p < 0.05$) different from the corresponding values in the air-exposed animals.
** Significantly ($p < 0.05$) different from the values immediately above and below.

Table 4. Maternal and fetal toxicity of IP-injected methyl isocyanate (MIC) in mice and rats.[a]

	Rats		Mice
	MIC, mg/kg		
Parameters	0	10	80
N	13	12	5
Maternal mortality, %	0	17	100
Surviving dams losing all embryos, *N*	0	2	—
Resorptions, mean	0.1	4.1 ± 2.6	—
Fetal body weight, g	3.9 ± 0.1	3.3 ± 0.4	—
Placental weight, mg	460 ± 14	416 ± 27	—

[a] The minimum volume of MIC that could be injected (2 μL/mouse) killed all the animals within 16 hr. All mice were pregnant and had 5–14 implantations. Values are mean ± SE.

trimester relative to those who were in their second or third trimester at the time of the accident could have been recorded if women mistook delayed menstruation or excessive vaginal bleeding as abortions. Because of the time gap, the survey could not ascertain the incidence of abortions in the 2 years preceding the Bhopal methyl isocyanate incident. Nevertheless, a 43% pregnancy loss in the postaccident period is three to four times higher than the normal incidence (as estimated by the Indian Council of Medical Research) of 6 to 10% abortions in Bhopal (*5*). Also, the rate of infant mortality within 30 days after birth is five to six times higher than that recorded for 1984 and 1983 (2 years preceding the accident).

In many ways, the experimental data confirm the clinical findings. For example, at 9 and 15 ppm MIC, more than 75% of mice lost all of their fetuses; however, in animals that did retain their pregnancy, the litter size was not dramatically reduced. This is unusual because with most agents that cause fetal toxicity, there is a noticeable increase in the loss of implantations, although most animals tend to retain some viable fetuses.

From this study, it is not clear if the fetal toxicity is due only to effects on the mother or due to both maternal and fetal effects. The greater body weight loss in the first 2 days after the exposure to MIC in pregnant animals that did not retain pregnancy as compared with those that did retain pregnancy indicates either an early onset of fetal toxicity or a relationship between marked maternal toxicity and ultimate fetal loss. The magnitude of hypoxia that results from these exposures (*8*, *12*) may not explain such marked fetal toxicity in light of the fact that in mice, marked hypoxia induced by other techniques was found to exert far less fetal toxicity than was observed in these other studies (*13*).

Fetal toxicity is probably caused by several factors operating concurrently. These could be general maternal and obligatory fetal hypoxia, changes in the placental blood flow, maternal and fetal hormonal changes, damage to the placenta, and possibly, direct effects on the fetus. Maternal hormonal changes, however, may be the result and not the cause of the fetal loss. The fact that fetal toxicity of MIC can be produced after IP injections indicates that pulmonary irritation is not essential for the toxicity. The similarity in the nature of fetal toxicity following inhalation or IP injections indicates the involvement of nonpulmonary factors in the fetal toxicity to MIC vapor, although the nonpulmonary factors may also signify nonspecific stress.

The epidemiological and experimental studies were equivocal as far as fetal malformations are concerned. In mice, there was no evidence of external malformations, although there was some evidence of increase in visceral anomalies. From the Bhopal reports, it appears that spina bifida is the most common fetal abnormality. If this anomaly is the result of inadequate skeletal formation, the observed decrease in the lengths of various bones of fetuses of MIC-exposed mice may be indicative of the clinical complications.

In summary, the data indicate that MIC exerts relatively selective fetal toxicity. The exact mechanism of this toxicity remains to be established.

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