METHYLISOCYANATE

This is a compilation of abstracts of articles identified during the preliminary toxicological evaluation of evidence on the developmental and reproductive toxicology of methylisocyanate. Methylisocyanate (CAS# 624-83-9) is used in the production of pesticides, polyurethane foam, and plastics. The public can be exposed through environmental releases, and occupational exposure can occur.

Compiled are abstracts from developmental and reproductive epidemiologic and animal toxicity studies and other relevant investigations. This information was used in a screen to select appropriate chemicals for presentation to the Developmental and Reproductive Toxicant Identification Committee as possible candidates for Committee consideration. The criterion for passing this screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. The epidemiologic studies report on developmental and reproductive sequelae related to accidental releases of methylisocyanate in Bhopal, India, in 1984. Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen.

- Seven epidemiologic studies of methylisocyanate reporting increased risk of adverse developmental or reproductive outcomes were identified, two of which were analytical studies of adequate quality. No epidemiologic studies reporting no increased risk of adverse developmental or reproductive outcomes were identified. Two related articles on the Bhopal release of methylisocyanate were identified.

- Six animal studies of methylisocyanate reporting reproductive or developmental toxicity were identified, as well as one animal study that did not report reproductive or developmental toxicity.

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I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

Long term morbidity in survivors of the 1984 Bhopal gas leak.
Cullinan P, Acquilla SD, Dhara VR.
Natl Med J India 1996;9:5-10.

BACKGROUND: The extent and nature of long term health sequelae among survivors of the Bhopal gas disaster are not known. In 1994 an International Medical Commission was set up with the aim of assessing respiratory, neurological and other health effects attributable to gas exposure. METHODS: An epidemiological survey of a representative sample of gas-exposed inhabitants of Bhopal was conducted in January 1994; for reference, a group of unexposed persons in the same city were surveyed. Questionnaires regarding health and exposure were administered to 474 persons, and a random sample (n=76) were subjected to respiratory and neurological testing. Responses to the questionnaire and the results of clinical testing were analysed according to a measure of individual gas exposure. RESULTS: A large number of subjects reported general health problems (exposed v. unexposed; 94% v. 52%) and episodes of fever (7.5/year v. 2.5/year); adverse outcome of pregnancy (e.g. still-births, 9% v. 4%) and respiratory symptoms (81% v. 38%), with a strong gradient by exposure category. This was not accounted for by differences in smoking, and was consistent with the results of spirometric testing. Neurological and psychiatric symptoms were reported more frequently by subjects in high exposure categories and the results of neurological examination and testing tended to confirm this finding. Ophthalmic symptoms demonstrated a similar pattern. Although a number of other symptoms were reported (with the possible exception of gastrointestinal disease), there was no clear evidence of other organ system damage attributable to gas exposure. CONCLUSION: The gradient of reported symptoms and clinical test results with estimates of exposure among these survivors of the gas leak suggests that a proportion of their current respiratory and neurological disease was due to gas exposure.

***Fetal loss and contraceptive acceptance among the Bhopal gas victims.**
Kapoor R.
Soc Biol 1991;38:242-8.

The rates of fetal loss and family planning acceptance among Bhopal gas victims from 1984 to 1989 were compared to those of a control group. In all, 136 eligible women in the affected area and 139 women in the control area were interviewed. Care was taken to ensure that these women had conceived at least once during the previous five years. The fetal loss rate among the gas-affected women was abnormally high (26.3 per cent) compared to that of women in the control area (7.8 per cent). Family planning acceptance in both areas was similar, with most women

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

using permanent methods. In the case of temporary methods, the percentage of use was higher in the gas-affected area.

Pregnancy complications in Bhopal women exposed to methyl isocyanate vapor.
Varma, D. R.
J A26[8], 1437-47. Environ Sci Health (A) 1991 Dec.

This study was done to determine the effects of the methyl isocyanate (MIC) spill from the Union Carbide pesticide plant at Bhopal on December 3, 1984 on the course of pregnancy. For this purpose, 2622 households within a radius of 1 km from the plant were surveyed in August 1986, and the health status of 200 children prenatally exposed to MIC was surveyed again in July 1990. A total of 638 women in 2622 households stated that they were pregnant at the time of the accident; 315 (49.4%) of these pregnancies did not culminate in live births. Pregnancy loss was higher in women who were in their first (58.8%) than in those who were in their second (42.1%) or third (40.1%) trimester of pregnancy during the MIC spill. Out of 323 live births, 12.1% (n=39) died between 1 and 30 days after birth and an additional 8 died between 1 and 18 months of age; the 1-30-day mortality rate was significantly greater than that recorded for the two years preceding the accident (2.7% in 1984 and 3% in 1983). Of the 273 surviving children born to MIC-exposed women, the status of 200 could be determined in 1990; 20 of these 200 had died before reaching the age of five years. According to their mothers, 122 out of 273 and 108 out of 200 children were sick in 1986 and 1990, respectively. The female-to-male ratio was 1.07 at birth but 1.8 in children who died. It is suggested that exposure to MIC resulted in a significantly higher pregnancy loss and neonatal mortality.

***Pregnancy outcome in women exposed to toxic gas at Bhopal.**
Bhandari NR, Syal AK, Kambo I, Nair A, Beohar V, Sexena NC, Dabke AT, Agarwal SS, Saxena BN.
Indian J Med Res. 1990 Feb;92:28-33.

A study was undertaken to compare the effects of exposure to the toxic gas in pregnant women in Bhopal with pregnant women in a similar, unexposed area. A high incidence of spontaneous abortions (24.2%) in the pregnant women exposed to the toxic gas was observed as compared to those in the control area (5.6%). Other indices of adverse reproductive outcome, such as the rate of still birth and congenital malformation were not found to be different. The perinatal and neonatal mortalities were significantly higher in the affected area (6.9 and 6.1% respectively), as compared to the control area (5.0 and 4.5% respectively).

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Morphological study of placentae of expectant mothers exposed to gas leak at Bhopal.
Kanhere S, Darbari BS, and Shrivastava AK.
Indian J Med Res 1987;86 Suppl(77-82).

Since there were a number of expectant mothers in the population affected by the Bhopal gas disaster in December, 1984, a study was undertaken on the morphology of human placenta in the women exposed at full term premature birth and after medical termination of pregnancy; 134 placentae were studied for evidence of any untoward effect of the gas (MIC) in the placenta. The mean weight of the placenta and foetal weight at full term were found to be lower in gas-exposed groups as compared to the controls, at full term. In premature births, the number of cases in the gas-exposed and control groups was too small to permit any statistical evaluation. In gross changes like like infarction and calcification, there was no significant difference between the groups. The incidents of fibrinold accrosis, syncytial knots, vasculosyncytial membrane was similar to data reported earlier in literature. Hydropic degeneration in the placentae of women who underwent medical termination of pregnancy was higher in the group exposed to gas, as compared to the controls. In full term deliveries, however, hydropic degeneration did not show any significant difference between the gas-exposed and the control groups.

Epidemiological and experimental studies on the effects of methyl isocyanate on the course of pregnancy.
Varma DR.
Environ Health Perspect 1987;72:153-7.

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.

Gynaecological and obstetrical survey of Bhopal women following exposure to methyl isocyanate.
Shilotri NP, Raval MY, Hinduja IN.
J Postgrad Med 1986;32:203-5.

A gynaecological examination and survey were carried out in 88 (Group I) symptomatic females staying ½ to 2 km away from Union Carbide Factory from where methyl isocyancate gas leaked out and 12 (Group II) symptomatic females staying 8-10 km away. Cervical smears were

examined from thirty-nine women who gave history of excessive vaginal discharge without local infection or pelvic factor accountable. Chief complaints were leucorrhoea (66 and 7 in groups I and II, respectively), irregular menses (16 and 3 in Groups I and II respectively) and menorrhagia (9 and 2 in Groups I and II respectively). Two cases showed dysplasia on cervical smear. Twenty nine out of thirty-eight cases who were known to be pregnant at the time of gas leak aborted immediately after the tragedy. Two had been delivered of pre-term babies with spina bifida, meningo-myelocele, limb deformities and signs of heart disease. The newborns died soon after birth and autopsy showed evidence of MIC lung.

B. Related articles

(There is no abstract for the research letter cited in the following two articles.)

Assessing Exposure to Toxic Gases in Bhopal

To the Editor: In their Research Letter about exposure to toxic fumes from the Union Carbide pesticide plant in Bhopal, India, Mr Ranjan and colleagues[1] found selective growth retardation in boys, but not in girls, who were either directly exposed or born to exposed parents. The assignment of exposure appears to be have been performed on an ecological rather than an individual basis, thus leading to the probability that some individuals (depending on their location on the night of the incident) might not necessarily have been exposed.

A study reported by the International Medical Commission on Bhopal (IMCB) found that some individuals living 6 to 8 km south of the plant who reported being exposed were, in fact, unexposed.[2] These authors were able to develop a quantitative individual exposure index for individuals exposed to gas on the basis of duration of exposure, location, and physical activity, and demonstrated associations between such individual exposure and subjective and objective measures of pulmonary function.

Such an exposure index is relatively easy to implement, and it would be of interest to test whether such an association between individual exposure and the growth retardation in adolescent boys would persist. Such an analysis may be important because some of the sample sizes in the study by Ranjan et al were relatively small.

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1. Ranjan N, Sarangi S, Padmanabhan VT, Holleran S, Ramakrishnan R, Varma DR. Methyl isocyanate exposure and growth patterns of adolescents in Bhopal. *JAMA.* 2003;290:1856-1857.
2. Dhara VR, Dhara R, Acquilla SD, Cullinan P. Personal exposure and long-term health effects in survivors of the Union Carbide disaster in Bhopal. *Environ Health Perspect.* 2002;110:487-500.

Assessing Exposure to Toxic Gases in Bhopal--Reply

Daya R. Varma, Nishant Ranjan, Satinath Sarangi, Steve Holleran, and Rajasekhar Ramakrishnan

JAMA. 2004;291:422-423.

In response to Dr Dhara, all exposed individuals in our study lived in Kainchi Chhola township, facing the Union Carbide plant toward the northeast and within 1 km of the plant--not 6 to 8 km south of the plant as he suggests. We had previously found a 43% rate of pregnancy loss in 3270 families[1] and 568 deaths in 2622 households[2] in this and in adjoining townships. Exposure to gases was ascertained by checking documents such as family ration cards used in December 1984, reference sheets of the survey carried out in early 1985 by the Tata Institute of Social Sciences, and medical records mentioning exposure-related symptoms, as well as verification by members of the local Health Committee. It was assumed that children aged 2 years or younger would have been near their mothers, and thus that postnatal exposure of children could be inferred from their mothers' exposure.

Recall bias is always a problem in retrospective studies, and especially so with a disaster of horrific magnitude such as that which took place in Bhopal. We believe that using an objectively determined categorical variable (yes/no for exposure) is preferable to quantifying the amount of exposure through a questionnaire. We agree that some subgroups in our study are small; we are presently undertaking a larger study to confirm and extend our findings.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

Teratological studies on methylisocyanate in Charles Foster rats (part - II).
Singh, RK; Srivastava A; Sethi N, and Dayal R.
Biol Mem. 1996; 22(1):21-5.
Charles Foster female rats were exposed to 0.212, 0.265 and 0.353 ppm of Methyl Isocyanate (MIC) vapours and mated with normal males of same strain. Teratological anomalies like wrist drop, everted claw, valgus deformity, syndactyly, blood clot formation, liver enlargement, cleft palate formation and unequal ribs were observed in the foetuses.

Methyl isocyanate induced morphological changes in the seminiferous epithelium of rats maintained on normal or protein deficient diets.
Bose M; Vachhrajani KD; Jha BS, and Dutta KK.
Bull Environ Contam Toxicol. 1994; 52(5):656-61.

[No abstract. Last paragraph of report: "Present studies suggest that MIC exposure cause reversible testicular damage and deficiency of protein may potentiate the effect of MIC within the frame work of these experiments."]

Dissociation between maternal and fetal toxicity of methyl isocyanate in mice and rats.
Varma DR; Guest I; Smith S, and Mulay S.
J Toxicol Environ Health. 1990; 30(1):1-14.

The contribution of maternal hormonal changes and pulmonary damage on the fetal toxicity of methyl isocyanate (MIC) was studied in mice and rats. Exposure to MIC decreased maternal plasma progesterone levels in mice that lost but not in mice that retained pregnancy. Fetal toxicity of MIC was not related to changes in maternal plasma corticosterone levels. Neither chronic administration of progesterone nor the suppression of pulmonary edema with dexamethasone decreased fetal toxicity of MIC. Embryos exposed in utero or in vitro to MIC vapor exhibited a concentration-dependent decrease in growth in culture. An acute dose (3 mmol/kg) of the MIC metabolites (methylamine, dimethylamine, trimethylamine, dimethyl urea) did not exert fetal toxicity. These data suggest that the fetal toxicity of MIC is partly independent of maternal toxicity and may result from its transfer across the placenta and interaction with fetal tissues.

Effect of subacute exposure to methyl isocyanate on testicular histomorphology in mice.
Arora U and Vijayaraghavan R.
Indian J Exp Biol. 1989; 27(4):347-9.

Mice exposed to methyl isocyanate (MIC; 134 mg.m-3 for 30 min = 4020 mg.min-1.m-3)

showed a marked loss of body weight after 24 hr and the mean body weight of the exposed group was significantly less than the control, even 15 days after the exposure. No significant change was observed on relative testicular weight. Spermatozoa in the seminiferous tubules disappeared 3 days post exposure. Primary and secondary spermatocytes were hypertrophied. Normalization occurred after 15 days.

Methyl isocyanate: reproductive and developmental toxicology studies in Swiss mice.
Schwetz, BA; Adkins B Jr; Harris M; Moorman M, and Sloane R.
Environ Health Perspect. 1987; 72:149-52.

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.

Reproductive toxicity of methyl isocyanate in mice.
Varma DR; Ferguson JS, and Alarie YJ.
Toxicol Environ Health. 1987; 21(3):265-75.

The effects of methyl isocyanate (MIC) vapor on pregnancy and fertility were studied in mice in view of the reported increase in reproductive complications in Bhopal following the December 3, 1984, accident. The whole-body exposure of mice to 9 and 15 ppm MIC for 3 h on d 8 of gestation led to resorption of greater than 80% of implants. In more than 75% of MIC-exposed animals, all implants were lost. At these concentrations, MIC did not cause external malformations. However, there was evidence of an increase in visceral abnormalities and a decrease in fetal and placental weights and in fetal skeleton sizes. MIC disturbed the estrus cycle and decreased the mating and pregnancy rate of female mice. The mating performance of MIC-exposed male mice was also decreased. Exposure to MIC increased serum corticosterone levels of male and nonpregnant female mice. MIC exerted no significant effects on serum corticosterone and progesterone levels of pregnant mice if the pregnancy was retained but caused a significant decrease in the serum levels of these two hormones in animals that lost all the implants. These studies show that the effects of MIC in mice mimic many of the reproductive complications in Bhopal. The mechanism of the reproductive toxicity of MIC remains to be identified.

B. Studies reporting no developmental or reproductive toxicity

Inhalation toxicity of methylisocyanate: assessment of germ cell mutagenicity and reproductive effects in rats.
Agarwal DK and Bose M.
Indian J Exp Biol. 1992; 30(6):504-8.

Adult male Wistar rats were exposed to methylisocyanate (MIC, 3.2 mg/l, single inhalation exposure for 8 min under static condition) or ethyl methanesulphonate (EMS, 150 mg/kg, single ip dose) for the assessment of germ cell mutagenicity and reproductive effects. Sequential matings of treated males with normal females on days 1-7, 8-14 and 15-21 post-exposure did not indicate any induction of dominant lethal mutation (increased frequency of preimplantation losses and early fetal deaths) by MIC but it was significantly induced by EMS as compared to respective controls. Males, necropsied after 21 days of exposure, showed no effect of MIC on epididymal sperm density and morphology. EMS also had no effect on sperm density but it significantly induced morphological abnormalities in sperm as compared to untreated controls. There was an acute and transitional reduction in reproductive performance (10-21%) of MIC-exposed males during days 1-14 post-exposure followed by recovery to the normal level during days 15-21 post-exposure. The progeny of MIC-exposed males was also normal in terms of litter size, litter weight, neonatal survival and body weight gain in litters up to 10 days post-partem. It is concluded with the evidence at hand that the observed failure of MIC to cause germ cell mutagenicity is related to its poor biodistribution to the target site(s) and a transient reduction in the reproductive performance of MIC-exposed males is a result of general stress and disconsolate copulation.