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Maternal Toxicity of Methacrylonitrile in Sprague-Dawley Rats

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Methacrylonitrile (MeAN) is used in the preparation of plastics, elastomers, homo- and copolymers and coatings (Windholz, 1983; Sax and Lewis, 1987) and as a chemical intermediate in the preparation of numerous organic chemicals (Windholz, 1983). MeAN is also used as a replacement for acrylonitrile in the manufacture of carbonated beverage containers (Cosidine, 1974) and is identified as a component of the main stream smoke of unfiltered cigarette made from air-cured, flue-cured, or a blend of these tobaccos (Baker et al., 1984). Occupational exposure to MeAN has also been reported (ACGIH, 1986; Amoore and Hautala, 1983).

MeAN is highly toxic in mice, rats and rabbits by dermal, respiratory and oral routes of administration (McOmie, 1949; Smyth et al., 1962). It has been shown to release cyanide ions into blood of rats and rabbits and its effects are diminished by standard therapy for cyanide poisoning (Pozzani et al., 1968; Tanii and Hashimoto, 1984). Human olfactory exposure studies have shown that MeAN vapors have very poor warning properties and the studies on response of rats and dogs to repeated MeAN vapors lead to the conclusion that humans should not be allowed to inhale more than 3 ppm vapor for 8 hours per day, five days per week (Pozzani et al., 1968). Studies in our laboratory showed that MeAN is a potent neurotoxin (Cavazos, et al., 1989) and that it depletes glutathione (GSH) both in vivo and in vitro (Day et al., 1988). Recently Farooqui et al., 1990a,b) have reported the tissue distribution and characterization of MeAN metabolism to cyanide.

Thousands of individuals are exposed to various nitriles including MeAN, during their manufacture, processing or disposal. A review of the scientific literature on MeAN reveals a lack of information concerning its reproductive toxicity. The purpose of this study was to assess the potential reproductive toxicity of MeAN in rats given the compound by gavage. The oral administration was chosen because trace amounts of untreated MeAN monomer may migrate into food and beverages that are packaged in containers made of MeAN copolymers.

MATERIALS AND METHODS

MeAN (purity >99%) was obtained from Aldrich Chemical Co., Milwaukee, WI. Adult female and male Sprague-Dawley rats were obtained at an initial body weight of 140-145 grams from Harlan Sprague-Dawley Inc., Indianapolis, IN. Animals were acclimatized in our animal facility for one week prior to experimentation at a constant temperature (20 ± 2° C) room with 12 hour light and dark cycles and fed commercial diet Purina Lab Chow (Purina Mills Inc., St. Louis, MO) with free access to tap water. The rats were then allowed to mate for four days and the day on which sperm were found in the vaginal smear was considered day

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0 of pregnancy according to Murray et al., (1978).

The protocol for the treatment of animals was essentially that of Murray et al., (1978). Groups of 6 pregnant female rats received MeAN in safflower oil as the vehicle in accordance with the following schedule: Group I received 50 mg/kg/day (0.25 LD50) MeAN during first week of gestation, Group II received 50 mg/Kg/day (0.25 LD50) during second week of gestation and Group III received 100 mg/Kg/day (0.5 LD50) during second week of gestation. The volume of the test material that was received by each animal was adjusted daily according to each animal's body weight on the day of treatment. The selection of dose of MeAN for this study was based upon the signs of toxicity observed in Sprague-Dawley rats following MeAN administration at various levels (Pozzani et al., 1968; Farooqui et al., 1990a).

The study was carried out in three parts as indicated by dosages (groups I-III). In group I, first week of gestation was chosen in order to examine the problems that could arise in early pregnancy upon exposure to MeAN; in group II, second week of gestation was chosen to examine the effect of MeAN on maintenance of pregnancy and in group III, the pregnant rats received 0.5 LD50 MeAN in second week of gestation. The dose was increased to assess any dose response and abnormal maternal behavior that may be experienced during pregnancy.

Animals were observed daily for physical signs of toxicity. The severity of signs were graded on a scale of $1<2<3<4$ (Ahmed and Farooqui, 1982). Since an abnormal change in body weight of pregnant animals is an important indicator of proper maintenance of pregnancy and embryonic growth (Murray et al., 1978) the control and MeAN ingested animals were weighed on designated days throughout the study period. Litter size, an important indicator of the effect produced by exposure to potentially hazardous chemicals, was also observed and recorded in all of the control and MeAN treated rats. The pregnant rats, at the end of the experiments, were sacrificed under diethyl ether anesthesia; their abdominal walls were cut open and the fallopian tubes and uteri were carefully observed for any morphological or physiological abnormalities including edema and the occurrence of any abnormal growth. The severity of the abnormalities were graded as described for signs of toxicity.

All data were calculated and expressed as mean $\pm$ SD of 6 animals. Statistical analysis was accomplished with Student's t-test.

RESULTS AND DISCUSSION

Within one hour following MeAN ingestion, the rats developed dose related mild to severe conditions including ataxia, trembling, convulsions, salivation and irregular breathing. The rats recovered from these signs at various times depending on the dose given.

The body weight of the pregnant rats was a varying factor in control and treated rats (Figure 1). The control rats had a normal body weight gain pattern throughout the gestation period. The mean weight of control rats was 198 g on gestation day 1 and by the 20th day ofgestation it was 255.6 g, a mean weight gain of 57.6 g. In contrast the mean weight of group I rats was 196 g on gestation day 1 which increased only up to 218.8 g by the 20th day of gestation, a mean weight gain of only 22.8 g. Initially the treated rats gained weight steadily for the first 15 days of gestation but not to the extent of controls over the same time period and then they started to lose weight.

The growth in the group II rats was slower than both the controls and group I animals. The mean weight of group II rats on gestation day 6 was 208.8 g and by the 15th day of gestation it was 212.3 g, a mean weight gain of 3.5 g as compared to 24.3 g over the same time period in control animals. The mean weight of group III rats was 206.9 g on gestation day 6 increasing only up to 212.3 g on the 15th day of gestation, a mean weight gain of only 5.4 g as

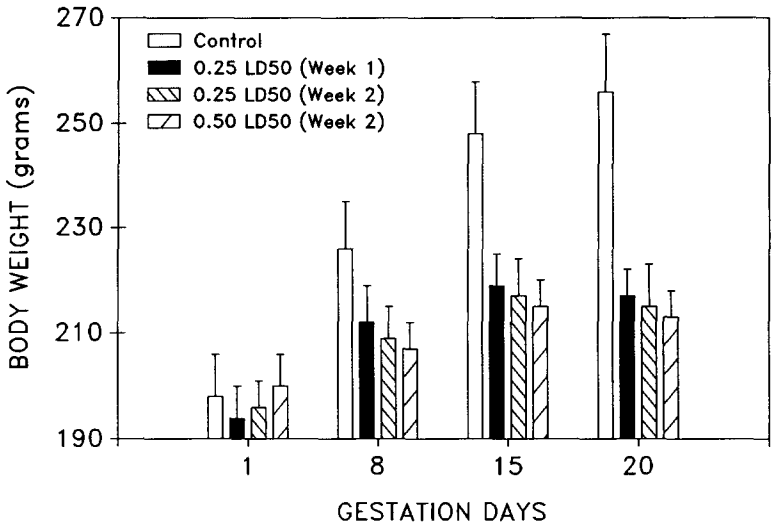

Figure 1. Body weight gain in pregnant Sprague-Dawley rats given MeAN. Group I received 0.25 LD50 MeAN (50 mg/Kg/day) during first week of gestation, group II received 0.25 LD50 MeAN (50 mg/Kg/day) during second week of gestation and group III received 0.5 LD50 MeAN (100 mg/Kg/day) during second week of gestation. Each bar represents mean ± SD of 6 animals. All treatments are significantly different from controls at $p < 0.01$.

compared to 24.3 g for the controls over the same time period. Weight loss was consistently observed in all the treated rats following the initial dosing.

The litter size of the pregnant rats given various doses of MeAN on scheduled gestation days is shown in Table 1. None of the group I and III rats delivered. Only one of the group II rats delivered a litter of 9 offspring whereas all the control rats delivered normal size litters on the 20th day of gestation.

Table 1. Litter data of pregnant rats given MeAN.

Treatment	No. deaths/No. dosed	Rats Delivered (%)	Litter size
Control	0/6	100	12.0 ± 1.7
Group I	0/6	0*	0.0 ± 0.0*
Group II	0/6	16*	1.5 ± 0.2*
Group II	1/6	0*	0.0 ± 0.0*

Experimental conditions are the same as described for Figure 1. All data are represented as mean ± SD of 6 animals, *$p < 0.01$.

Table 2 shows the incidence of edema and release of fluids in the maternal reproductive tract of MeAN ingested rats. Three out of five treated rats (60%) in group I animals exhibited mild to severe edema in the fallopian tubes. In group II and III, 4 out of 6 treated rats (66%) exhibited severe edema in the fallopian tubes.. A significant observation in group III animals, in addition to severe edema in fallopian tubes, was the occurrence of a globular structure in one of the fallopian tubes of 1 out of 6 treated rats (17%). None of the control

Table 2. Incidence of edema and release of fluids in the fallopian tubes of pregnant rats given MeAN.

Treatment	Edema and fluids in fallopian tubes
Control	0.5 ± 0.0
Group I	1.4 ± 0.2 (280)*
Group II	3.2 ± 0.4 (640)*
Group III	3.7 ± 0.5 (740)*

Experimental conditions are the same as in Figure 1. Severity of edema was graded on a scale of 1<2<3<4 (Ahmed and Farooqui, 1982). All data are represented as mean ± SD of 6 animals, *$p < 0.01$. Numbers in parentheses are percent of controls.

rats showed edema in the maternal reproductive tract comparable to that in MeAN treated rats.

The results of these studies indicate a potential for MeAN to interfere with maintenance of pregnancy and the initial embryonic development in Sprague-Dawley rats. Oral administration of 0.25 LD50 MeAN (50 mg/Kg/day) during the first week of gestation caused the rats to abort early in pregnancy. This effect could be attributed to either the whole molecule of MeAN or any of its possible metabolites including cyanide. Willhite et al. (1981) have reported such an effect produced by the cyanide released from acrylonitrile. Cavazos, et al. (1989) and Farooqui et al. (1990a) have reported that substantial amount of an oral dose of MeAN remains in the body of young Sprague-Dawley for several days. This residual pool of MeAN may be further metabolized to cyanide and exhibit the effect seen in this study. Recently, acetone has been reported as a new metabolite of methacrylonitrile (Ghanayem et al., 1991). The role of acetone in MeAN toxicity remains to be established.

GSH plays an important role in the biochemical and physiological functions of normal cells. Conjugation of GSH with xenobiotics is one of the primary mechanisms of detoxication (Kosower, 1976). When GSH is depleted, as it has been demonstrated following MeAN ingestion, the entire molecule or any of its metabolites may find access to the target organs, uteri and fallopian tubes in this study, causing maternal toxicity.

While in groups I and III none of the dosed rats delivered, 1 outof 6 dosed rats in group II delivered normal size litter. This suggests MeAN induced variation in the gestation period in these animals. Severe weight loss, especially with higher dose of MeAN, may have played a major role in the spontaneous abortions that have occurred in our study. Such a weight loss is stressful and detrimental to the survival of embryos.

The incidence of edema in the fallopian tubes and the occurrence of a globular structure following MeAN ingestion are significant observations of our study. Edema in fallopian tubes could be the result of possible cellular damage in the epithelial lining of fallopian tubes releasing fluids. Cellular damage and ulceration have been reported following exposure to other aliphatic nitriles identical in structure to MeAN (Ghanayem and Ahmed, 1982; Szabo and Seyle, 1972). Alternatively, the fluid found in the fallopian tubes may have resulted from the lysis of developing embryos. Since we could not examine and evaluate the nature of the globular structure, it is possible that it could be an embryo or a fetus resorpting into the tubular epithelium as a result of MeAN ingestion. These observations suggest that prolonged chronic exposure to MeAN may lead to tumorigenic and teratogenic effects if pregnancies are maintained. So far there are no reports in the literature concerning MeAN to substantiate these effects.

In conclusion, MeAN exhibits a potential for maternal toxicity in Sprague-Dawley rats.

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REFERENCES

ACGIH (1986) Documentation of the threshold limit values and biological exposure indices, American Conference of Governmental Industrial Hygienists, 5th ed. p 370., Cincinnati, OH.

Ahmed AE and Farooqui MYH (1982) Comparative toxicities of aliphatic nitriles. Toxicol Lett 12:157-163.

Amoore JE and Hautala E. (1983) Odor as an aid to chemical safety: Odor threshold compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution J Appl Toxicol 3:272-290.

Baker RR, Dymond HF and Sillabeer PK (1984) Determination of α , β -unsaturated compounds formed by burning cigarette. Anal Proc 21:135-137.

Cavazos R, Farooqui MYH, Day WW, Villarreal MI and Massa E (1989) Disposition of meth-acrylonitrile in rats and distribution in blood components. J Appl Toxicol.9:53-57.

Cosidine DM (1974) Chemical and Process Technology Encyclopedia. pp. 30-34, McGraw-Hill Book Co., New York, NY.

Day WW, Cavazos R and Farooqui MYH (1988) Interaction of methacrylonitrile with glutathione. Res Commun Chem Pathol Pharmacol 62:267-278.

Farooqui MYH, Cavazos R, Villarreal MI and Massa E (1990a) Toxicity and tissue distribution of methacrylonitrile in rats. Ecotoxicol Environ Safety 20:185-196.

Farooqui MYH, Diaz R and Cavazos R (1990b) Metabolism of methacrylonitrile to cyanide - in vitro studies. J Biochem Toxicol 5:109-114.

Ghanayem BI and Ahmed AE (1982) Acrylonitrile induced gastrointestinal hemorrhage and the effects of metabolism modulation in rats. Toxicol Appl Pharmacol 68:290-296.

Ghanayem BI, Sanchez I and Burka LT (1991) In vivo biotransformation of methacrylonitrile in male F344 rats. The Pharmacologist 33, 159.

Kosower EM (1976) Chemical properties of glutathione. In: Glutathione Metabolism and Function. Eds. I.M. Arias and W.B. Jacoby. Raven Press, New York.

McOmie WA (1949) Comparative toxicity of methacrylonitrile and acrylonitrile. J Ind Hyg Toxicol 31:113-121.

Murray FJ, Schwetz BA, Nitschke KD, John JA, Norris JM and Gehring PJ (1978) Teratogenicity of acrylonitrile given to rats by gavage and inhalation. Food Cosmet Toxicol 16:547-551.

Pozzani UC, Kinkead ER and King JM (1968) The mammalian toxicity of methacrylonitrile. Am Ind Hyg Assoc J 29:201-210.

Sax NI and Lewis RJ (1987) Hawley's Condensed Chemical Dictionary. 11th ed., p. 751, Van Nostrand Reinhold Co., New York, NY. (1987)

Smyth Jr. HF, Carpenter CP, Weil CS, Pozzani UC and Striegel JA (1962) Range finding in toxicity data. Am Ind Hyg Assoc J 23:95-99.

Szabo S and Seyle H (1972) Duodenal ulcers produced by propionitrile in rats. Arch Toxicol 93:390-394.

Tanii H and Hashimoto K (1984) Studies on the mechanism of acute toxicity of nitriles in mice. Arch Toxicol 55:47-54.

Willhite CC, Ferm VH and Smith RP (1981) Teratogenic effects of aliphatic nitriles. Teratology 23:317-323.

Windholz M (1983) Merck Index: An Encyclopedia of Chemicals and Drugs. Methacrylonitrile. p. 774. Merck & Co., Inc., Rahway, N.J. (1983)

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