Bull. Environ. Contam. Toxicol. (1986) 36:337–341

Environmental Contamination and Toxicology

Some Effects of Multiple, Sublethal Doses of Monosodium Methanearsonate (MSMA) Herbicide on Hematology, Growth, and Reproduction of Laboratory Mice

J. A. Prukop and N. L. Savage

Department of Biology, Pan American University, Edinburg, TX 78539

Monosodium Methanearsonate (MSMA) is an organic aresenical herbicide used as a selective herbicide for the postemergence control of crabgrass, Dallisgrass and other weedy grasses in turf and for the control of Johnsongrass, nutsedge, watergrass, sandbur, cocklebur, pigweed and grasses in cotton and noncrop areas. The mode of action by which MSMA and similar organic arsonates kill plants seems to be related to a disturbance of phosphorus metabolism (Committee on Medical and Biologic Effects of Environmental Pollutants 1977).

Very few effects of MSMA on animals have been reported. Oral lethal doses vary widely depending on the animal model and include 1800 mg/Kg for white mice (Dickinson 1972), 1200-1600 mg/Kg for cattle when applied at 10 mg/Kg/day (Dickinson 1972), 346 mg/Kg for snowshoe hares (Exon et al. 1974), and 600 mg/Kg for white-footed mice (Peromyscus leucopus) (Judd 1979). Sublethal MSMA concentration effects reported include depressed hematocrits and blood glucose levels (Judd 1979) and altered female nest building behavior (Lopez and Judd 1979), both effects in the white-footed mouse. The mechanisms causing these lethal and sublethal effects are unknown.

The purpose of these studies was to determine if multiple, sublethal doses of MSMA affected hematology, growth, reproduction and survival of laboratory strains of mice. The results of these investigations indicated that the doses used, 11.9 or 119 mg MSMA/Kg body wt, administered orally three times per week for 10 weeks had no effect on hematology, growth or survival of laboratory mice but apparently decreased reproductive capabilities of males and altered reproductive behavior of females.

MATERIALS AND METHODS

Harlan ICR Swiss mice (Harlan Industries, Indianapolis, Indiana) were housed in plastic cages and provided Purina Laboratory Chow and water ad libitum. Animal rooms were maintained at 23° ± 2° C with 12 hours light and 12 hours dark.

Ansar 529 H.C. (Ansul Company, Marinette, Wisconsin) commercial formulation of MSMA was diluted in physiological saline (0.85% NaCl) prior to administration of doses equal to 0.01 LD_{50} or 0.1 of LD_{50} as reported by manufacturer. These doses were 11.9 and 119

mg MSMA/Kg body wt, respectively.

Animals previously maintained in the laboratory for at least 2 weeks to allow adjustment and to ensure that they were free of disease were separated into groups. The groups consisted of a control receiving no treatment, a control receiving oral doses of saline and two experimental groups, one receiving 11.9 mg/Kg and the other receiving 119 mg/Kg. Treatment consisted of oral intubation of herbicide every other day for a period of 10 weeks. Each cage contained initially one male per five females, and males were removed after 19 days. Pregnant females were also separated as pregnancy became obvious. Animals were weighed initially and periodically thereafter. The numbers of pregnancies were recorded and the numbers of animals per litter recorded. Terminal hematocrits, total and differential blood cell counts, total serum proteins and serum protein fractions were determined for the adult mice.

Blood was collected by cardiac puncture on anesthetized animals and placed in heparinized tubes. Total erythrocyte and leukocyte counts were made with a hemacytometer. Differential counts were made on Wright's stained smears. Hematocrits were determined on an Adams micro-hematocrit reader. Total serum protein concentrations were determined in a Beckman ACTA III C Spectrophotometer at 750 nm using the Folin-Ciocalteu phenol reagent (Lowry et al. 1951). A standard concentration curve was determined for known quantitites of normal range serum proteins (Dade, Moni-Trol I). Absorbance values of unknown samples of serum protein were compared to the standard curve to determine their concentrations.

Serum protein fraction concentrations (albumin, α, β, and γ globulins) were determined on samples separated by electrophoresis on cellulose polyacetate strips, stained with Ponceaus S and eluted with 0.1 N NaOH prior to measurement of absorbance (Bradshaw 1966).

Forty, 49 day old, Harlan/ICR Swiss mice were placed in two groups of 20 mice each. Mice were housed two to a cage, one male and one female. Females received no treatment. Males were dosed every other day for 19 days on a 3 day per week basis. Males in Group A received physiological saline in a volume of 0.01 ml/5 g body weight and males of Group B received a similar volume of 119 mg MSMA/Kg body wt. Males were placed with females on the first day of dosing and separated after 19 days. Male fertility was indicated by the incidence of pregnancy.

Chi-square without a priori hypothesis was used to determine significant differences in male fertility, litter frequency, and still birth frequency at both the 0.05 and 0.01 levels. Student's t-test was used to compare weight gains between groups. Analyses of variance were performed on growth rates and blood parameters, and the F-test was used to determine any significant difference between the experimental groups and the controls (Schefler 1969).

RESULTS AND DISCUSSION

We found no significant difference in total erythrocyte counts, hematocrits, total or differential leukocyte counts, total serum proteins or serum-protein fractions between control and herbicide-treated mice.

A significant difference was found for weight gain ($P<0.01$) and litter frequency in mice treated with 119 mg MSMA/Kg body wt for 10 weeks. Untreated control mice gained 12.5 ± 2.7 g, saline-treated control mice gained 10.4 ± 1.8 g, 11.9 mg MSMA/Kg treated mice gained 9.1 ± 3.6 g and 119 mg MSMA/Kg treated mice gained 6.3 ± 1.9 g. One mouse in this group died. This difference in weight gain can be attributed to the fact that none of the females given 119 mg MSMA/Kg body wt produced litters. Eighty percent of untreated control mice, 100% of saline treated control mice and 50% of the 11.9 mg MSMA/Kg body wt treated group produced litters. The mean weight gain of females from this 11.9 mg group that produced litters was 11.9 ± 2.2 g while the mean weight gain for those females not producing litters was 5.4 ± 0.8 g indicating that pregancy produced a significant weight gain ($P<0.005$).

Females that failed to produce litters in the 11.9 mg MSMA/Kg group had been mated with the same male. Since both males and females received equivalent doses of herbicide, it was impossible to determine whether the male or the female (or both) had been affected. Therefore, 10 pairs of mice were established in which the males received a dose of 119 mg MSMA/Kg body wt on an alternate day basis and the females were not treated. Fifty percent of the females in the treated group produced litters while 90% of the females in the saline control group produced litters ($P<0.05$).

There were no significant differences among control and experimental groups in litter size, litter weight at 3 weeks of age or still birth frequency.

Treatment of male with either dose of MSMA apparently reduced reproductive capacity in those mice. This antifertility effect may be the result of any number of factors including alteration of spermatogenesis, sperm motility, or mating behavior. Pasi et al. (1974) reported that the herbicides diquat and paraquat induced antifertility effects at different maturation stages of spermatogenesis. Sperm motility might also be affected since arsenic affects generation of ATP (Berry et al. 1974) and ATP is necessary for sperm motility.

Lopez and Judd (1979) reported a reduction in nest building activity of female P. leucopus treated with MSMA but no effect on male behavior. A possible modification of the maternal instinct in two females out of four that had been dosed with 119 mg MSMA/Kg and succeeded in producing litters in our study was noted. The affected females did not build a nest, rarely huddled over the young, or retrieved them when they were separated from the young, all of which are maternal instincts (Green 1966). The young mice from these two females died within 2 to 3 days after birth. A slight depression in

the reproductive success and maternal instinct over a long period could produce significant evolutionary changes (Ferguson 1972).

Hematologic parameters of MSMA treated mice were apparently not affected under the conditions of this investigation. Earlier reports (Judd 1979) indicated a decrease in MSMA treated P leucopus hematocrits, hemoglobin concentration and blood glucose levels. Variability in results from different studies is not surprising since different procedures and experimental animals are involved. Variability is very evident in acute toxicites (500 mg MSMA/Kg body wt, for P. leucopus; Judd 1979; 1800 mg/Kg for white mice, Dickinson 1972) and might be anticipated for other parameters as well.

Acknowledgement. This study was supported in part by N.I.H., Division of Research Resources, Minority Biomedical Research Support Program Grant RR08038. The data presented here represent a portion of the thesis submitted by J.A. Prukop to Pan American University, Edinburg, Texas, in partial fulfullment of the requirements for the M.S. degree.

REFERENCES

Berry JW, Osgood DW, St. John PA (1974) Chemical villains: a biology of pollution. C.V. Mosby Company, Saint Louis, Missouri.

Bradshaw LJ (1966) Introduction to molecular biological techniques. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Committee on medical and biologic effects of environmental pollutants (1977) Medical and biologic effects of environmental pollutants. Arsenic. National Academy of Sciences, Washington, D.C.

Dickinson JO (1972) Toxicity of the arsenical herbicide monosodium acid methanearsonate in cattle. Amer J Vet Res 33:1889-1892.

Exon JH, Harr JR, Claeys RR (1974) The effects of long term feeding of monosodium acid methanearsenate (MSMA) to rabbits. Nutr Rpts Internat 9:351-357.

Ferguson DE (1972) The new evolution. Environment 14:30-35.

Green EI (1966) Biology of the laboratory mouse. McGraw-Hill, New York, New York.

Judd FW (1979) Acute toxicity and effects of sublethal dietary exposure of monosodium methanearsonate herbicide to Peromyscus leucopus (Rodentia: Cricetidae). Bull Environ Contam Toxicol 22:143-150.

Lopez JF, Judd FW (1979) Effect of sublethal dietary exposure of monosodium methanearsonate herbicide on the nest building behavior of the white-footed mouse, Peromyscus leucopus. Bull Environ Contam Toxicol 23:32-32.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265-275.

Pasi A, Embree JW, Eisenlord GH, Hine CH (1974) Assessment of the mutagenic properties of diquat and paraquat in the murine dominant lethal test. Mut Res 26:171-175.

Peoples SA (1975) Review of arsenical pesticides. In: Woolson EA (ed) Arsenical pesticides. American Chemical Society Symposium Series 7, Washington, D.C. p.1.
Schefler WC (1969) Statistics for the biological sciences. Addison-Wesley, Menlo Park, California.

Received March 23, 1985; accepted April 1, 1985.