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Acute Toxicity and Effects of Sublethal Dietary Exposure of Monosodium Methanearsonate Herbicide to *Peromyscus leucopus* (Rodentia: Cricetidae)

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Arsenic is a ubiquitous element that is present in air, water, soil and living tissues. Depending on the concentration a given arsenical compound may show no action, be toxic, or stimulatory (PEOPLES 1975). Arsenical pesticides have been widely used in agriculture for more than a century and they represent the single largest manmade input of arsenic into the environment (SANDBERG and ALLEN 1975). Monosodium methanearsonate (MSMA) is used extensively as a selective post-emergent herbicide in cotton and non-crop areas. It is water soluble and contains arsenic in the pentavalent form. The oral LD_{50} to white mice and rats is 1,800 and 700 mg/Kg, respectively (DICKINSON 1972); however, DICKINSON (1972) found that a total dose of 100 mg/Kg administered at a rate of 10 mg/Kg/day was lethal to cattle. EXON et al. (1974) reported an LD_{50} of 173 mg/Kg for snowshoe hares. Thus, there is a great deal of variation in acute toxicity among species. The objectives of this study were to determine the acute toxicity of MSMA herbicide to the native cricetine rodent Peromyscus leucopus and to assess the sublethal effects of MSMA herbicide on hematocrit, hemoglobin concentration and blood glucose concentration.

MATERIALS AND METHODS

Animals and Maintenance. Peromyscus leucopus used in the experiments were trapped at various localities in Hidalgo County, Texas or they were F1 offspring of field animals. Field animals were maintained in the laboratory on ad libitum rations of water and Purina Lab Chow for at least 21 days to ascertain their health and to eliminate pregnant females. Weights of animals ranged from 17.5 to 31.8 g. Mice were housed individually in plastic cages, 26 cm l x 21 cm w x 11 cm d, floored with wood shavings and topped with a perforated metal cover. Cotton was provided as nesting material. Room temperature was 22° to 24° C and photoperiod was 12L:12D.

Acute Toxicity. MSMA (in Ansar® 529 HC) was delivered in the drinking water. Five groups of mice received, respectively: 1) tapwater (control, N = 12), 2) 10,000 ppm herbicide in tapwater (N = 14), 3) 5,000 ppm herbicide in tapwater (N = 14), 4) 3,000 ppm herbicide in tapwater (N = 14), and 5) 1,000 ppm herbicide in

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tapwater (N = 10). Mice were housed separately as outlined above and drinking rates, food consumption rates, body weights and days to death monitored for each individual. Drinking rates were determined by weighing water bottles daily to the nearest .01 g. Loss due to evaporation was accounted for by subtracting the mean weight loss from two control bottles (inverted over empty cages) from each mouse's water bottle. Body weights were monitored by weighing the mice to the nearest .1 g every other day. Food consumption was determined by weighing each mouse's food ration daily to the nearest .01 g. Rates of food and water consumption were expressed as g/g body weight/day.

Acute toxicity of single oral doses delivered by intubation was also determined. Concentration was adjusted according to weight and doses were expressed as mg MSMA/Kg body weight. Volume was kept between .4 and .5 ml. Stepwise doses of herbicide at intervals of 50 mg MSMA/Kg were delivered to groups of 4 to 8 mice. Percent mortality at 7 days was recorded and LD_{50} was calculated by linear regression of mortality on dose with inverse prediction.

Sublethal Concentration Experiments. Body weights of 12 mice receiving 1,000 ppm herbicide and 10 mice receiving tapwater were monitored for 60 days. Means and variances were calculated initially, at 30 days and at 60 days elapsed time.

Hematocrit, hemoglobin concentration and blood glucose concentrations were measured in a group of 5 control animals (tapwater) and a group of 10 herbicide treated mice (1,000 ppm). All 15 mice received tapwater for 30 days, then they were separated into control and experimental groups for 30 days. Food was removed from the cages 12 hours prior to drawing blood. Blood was drawn from the posterior canthus of the eye in heparinized microcapillary tubes. Hematocrits were determined after centrifuging for 7 min at 10,000 rpm. Hemoglobin concentration was estimated from a sample of .02 ml of blood by the cyanmethemoglobin method using a Bausch and Lomb Spectronic 20 colorimeter at 540 μ. Blood glucose concentration was determined from whole blood by the glucose oxidase method using Worthington Biochemical Corporation reagents. Results were expressed as % packed formed elements (hematocrit), g/100 ml blood (hemoglobin concentration), and mg glucose/100 ml blood.

Statistical procedures employed are those of SOKAL and ROHLF (1969). Probability value less than .05 is considered significant.

RESULTS

Acute Toxicity. Single oral dosages greater than 500 mg MSMA/Kg were 100% lethal while dosages of 150 mg and below produced no mortality. The LD_{50} was 300 mg MSMA/Kg and the 95% confidence interval extended from 250 to 350 mg MSMA/Kg.

Table 1 provides results of the acute toxicity studies of herbicide in the drinking water of P. leucopus. Mice were

TABLE 1

Comparison of Mortality, Weight Loss, Fluid Consumption and Food Consumption Among Groups of *Peromyscus leucopus* Receiving Herbicide in Their Drinking Water and Controls (Tapwater)

Regimen	MSMA Concentration	Number of Animals	Percent Mortality	Days to Death		Mean % Wt. Lost		Mean % Fluid Consumed (g/g B.W./day)		Mean % Food Consumed (g/g B.W./day)	
	mg/ml			Range	$\bar{X}$	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
10,000 ppm	7.190	14	100.0	2 - 4	3.0	12.51	5.41	.031	.020	.034	.027
5,000 ppm	3.595	14	92.8	1 - 6	3.2	14.29	10.64	.061	.033	.046	.017
3,000 ppm	2.157	12	91.7	2 -20	9.2	19.68	15.05	.100	.042	.073	.045
1,000 ppm	0.719	10	0.0	--	--	Gained		.173	.044	.159	.022
Tapwater	--	12	0.0	--	--	1.37	1.00	.161	.047	.160	.022

maintained on the regimen for 21 days. The 3,000, 5,000 and 10,000 ppm solutions were over 90% lethal. All the controls and the mice on the 1,000 ppm regimen survived. Time to death, mean percent weight loss, and fluid and food consumption rates were inversely correlated with concentration over the 3,000 to 10,000 ppm range. There was no significant difference in the drinking and eating rates of the control and 1,000 ppm groups.

Because days to death, weight loss, and food and liquid consumption were inversely correlated with concentration of the toxic regimen, the possibility existed that the mice on these regimen died from dehydration and/or starvation due to unpalatability of the solutions rather than from the toxic effects of the herbicide. (It is well known that water deprived mice voluntarily reduce food intake.) If this was the case, then percent weight loss and days to death should be similar in deprived groups of mice. I tested this by placing one group of mice on ad libitum food but no water; another group received ad libitum water but no food; and both water and food were removed from a third group of mice. Days to death and percent weight lost at death were recorded for each mouse. Data in Table 2 show that the percent weight lost by mice in these experiments was markedly higher than that of mice on lethal herbicide regimen. The mean percent weight lost by deprived mice was more than double the mean percent weight lost by mice on the 5,000 and 10,000 ppm regimen and approximately double that of mice on the 3,000 ppm regimen. Also, most of the mice on the toxic regimen died sooner than deprived mice.

TABLE 2

Comparison of Elapsed Time to Death and Percent Weight Loss Among Food Deprived, Water Deprived, and Food and Water Deprived Groups of Peromyscus leucopus

Regimen	No. of Mice	Days to Death		Percent Wt. Lost /	
		Range	$\bar{X}$	$\bar{X}$	S.D.
Food but No Water	10	3-14	7.8	44.6	7.88
Water but No Food	5	4 - 7	5.0	31.7	8.54
No Water or Food	5	4 - 7	5.0	36.2	6.97

Examination of daily liquid consumption values showed that most of the mice on the lethal regimen consumed relatively large amounts of fluid on the first day and far less thereafter, which suggests that the toxicity was manifest within the first 24 hours of exposure. The possibility that concentration per se rather than effects of the herbicide was important in producing mortality was

also tested. A group of 9 mice were placed on NaCl solutions of increasing concentration. These mice maintained body weight while drinking a solution of 0.4 molar concentration, which is more than double the highest herbicide concentration. Thus, the conclusion that the mice died as a result of the toxicity of the herbicide appears compelling.

Table 3 provides a comparison among regimen of the amounts of MSMA consumed. I have used mean time to death and mean drinking rates in constructing this table, and I have assumed a mean weight of 21.0 g for the mice. Mice on the 10,000 and 5,000 ppm regimen consumed similar amounts of MSMA. Mice on the 3,000 ppm consumed about 3 times more MSMA and mice on the 1,000 ppm regimen consumed the largest total dosage of MSMA. These data suggest that toxicity is a function of the quantity of MSMA consumed in a given unit of time rather than to accumulated dose.

Sublethal Concentration Experiments. Mice receiving tapwater and 1,000 ppm herbicide (477 ppm MSMA) were continued on these regimen for 60 days. Mean body weights at initiation was 21.9 g (S.D. = 3.7 g) for 12 controls and 19.8 g (S.D. = 2.7 g) for 10 herbicide treated mice. At the end of 60 days mean weight of the 12 control mice was 21.6 g (S.D. = 3.8 g) and the average weight of the 10 herbicide treated mice was 20.9 g (S.D. = 2.9 g). There were no significant changes in body weight; thus, in subsequent experiments on sublethal effects a concentration of 1,000 ppm herbicide was used.

Effects of the 1,000 ppm regimen on hematocrit, hemoglobin concentration and blood glucose concentration are shown in Table 4. All mice received tapwater the first 30 days and then 10 mice were placed on 1,000 ppm herbicide for 30 days. Comparison of values between the first 30 days and second 30 days within the herbicide treated group shows the effect of the herbicide treatment. Comparison between time periods within the tapwater control provides data on the degree of change that may be expected. After 30 days, mice receiving herbicide in the drinking water showed a significant decrease in mean hematocrit ($t = 4.196$, 18 df, $P < .001$). Mean hemoglobin concentration of the experimental group was markedly lower than that of controls before being placed on the herbicide regimen, but hemoglobin concentration remained relatively constant. Sample size was 9 for the herbicide treated mice because I failed to obtain enough blood from one mouse to run all the tests. Mean blood glucose concentration was significantly lower after 30 days elapsed time for mice receiving herbicide ($t = 2.787$, 17 df, $P < .02$).

TABLE 3

Comparison of Drinking Rates and Amounts of MSMA Consumed Among Groups of *Peromyscus leucopus* Receiving Herbicide in Their Drinking Water

Regimen (ppm)	$\bar{X}$ Days to Death	$\bar{X}$ Drinking Rate (g/g B.W./Day)	$\bar{X}$ g (or ml) Fluid Consumed (g/g B.W./$\bar{X}$ Time to Death or 21 Days)	$\bar{X}$ g (or ml) Fluid Consumed by 21 g Mouse During $\bar{X}$ Time to Death or 21 Days	MSMA Conc. (mg/ml)	mg MSMA Consumed to Death or Criterion	mg MSMA Consumed/ Kg B.W.
10,000	3.0	.031	.093	1.953	7.190	14.0	667
5,000	3.2	.061	.195	4.095	3.595	14.7	700
3,000	9.2	.100	.920	19.320	2.157	41.7	1,986
1,000	-	.173	3.633	76.293	0.719	54.9	2,614
Tap water	-	.161	3.381	71.001	-	-	-

TABLE 4

Comparison of Hematological Parameters Between Control (Tapwater) and Herbicide Treated Groups of Peromyscus leucopus

Parameter	Time Period	Tapwater			1,000 ppm Herbicide		
		N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.
Hematocrit (%)	1st 30 days	5	46.8	2.6	10	46.3	3.7
	2nd 30 days	5	46.6	4.6	10	40.3	2.6
Hemoglobin Concentration (g/100 ml)	1st 30 days	5	12.0	0.8	9	10.7	1.6
	2nd 30 days	5	11.7	0.8	9	10.3	0.8
Blood Glucose Concentration (mg/100 ml)	1st 30 days	5	135.0	31.4	10	149.8	20.1
	2nd 30 days	5	131.0	34.2	9	124.3	19.7

DISCUSSION

These experiments show that MSMA herbicide is toxic to P. leucopus at relatively low concentrations compared to albino mice and rats and that white-footed mice voluntarily consume lethal concentrations. Although limited, the data suggest that there is great variability in susceptibility to MSMA toxicity among mammalian species (DICKINSON 1972, EXON et al. 1974, PEOPLES 1975).

Mice on the 1,000 ppm herbicide (477 ppm MSMA) regimen consumed the largest total amount of MSMA, but showed no mortality. This supports EXON et al.'s (1974) conclusion that MSMA toxicity is primarily dependent on the quantity of oral dosage received during a given time period. All of the mice that died in the acute toxicity experiments showed evidence of severe diarrhea, which is consistent with reports that symptoms of acute arsenic poisoning includes a violent hemorrhagic gastroenteritis leading to a profound loss of fluid and electrolytes, resulting in collapse, shock and death (DICKINSON 1972, SWIGGERT et al. 1972, WEBSTER 1941).

In the sublethal concentration experiments, lower hematocrits and absence of an accompanying decrease in hemoglobin concentration suggest decrease in erythrocyte size. MAHAFFEY and FOWLER (1977) reported increased numbers of erythrocytes, but reduced hemoglobin and hematocrits following administration of sodium arsenate to rats; which also suggests that cell size is decreased. Disturbance of electrolyte balance might cause a loss of water from the red cells resulting in smaller erythrocyte size. Lower blood glucose concentrations may reflect impaired liver function. EXON et al. (1974) reported that adult rabbits fed 50 ppm MSMA in the feed developed toxic hepatitis after 7 weeks' exposure. FOWLER et al. (1977) reported that rats receiving 40 and 85 ppm sodium arsenate in the drinking water for 6 weeks developed marked ultrastructural and biochemical changes in hepatocyte mitochondria. They suggested that

arsenic may produce differential damage to a number of cellular organelle systems and their attendant biochemical functions.

The manufacturer recommends mixing 3 quarts (2.84ℓ) of herbicide with 90 gallons (341ℓ) of water for use on cotton. This is a 8,333 ppm solution. A 21 g P. leucopus would have to consume about 3 ml of this solution, within 3 days, to be lethal. Thus, it is unlikely that mice would consume lethal quantities unless the herbicide was misused. However, it is possible that mice could consume enough herbicide to produce sublethal effects. Furthermore, populations stressed by environmental extremes such as high temperature or drought might be more susceptible to herbicide effects (SELBY et al. 1977). For example, FERM and KILHAM (1977) showed that the teratogenic effects of arsenic and hyperthermia were synergistic on hamsters. Thus, either accidental high exposure or chronic exposure to arsenic in the environment might have marked effects on P. leucopus and other mammalian populations.

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