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Acute Toxicity of Some Hydrazine Compounds to Salamander Larvae, *Ambystoma* spp.

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Although hydrazine compounds have been used extensively by industry for a very long time, they have become important in recent years as propellants for aerospace operations. As a consequence they have been investigated by both the military and industry in terms of their potential to contaminate the environment as well as their toxicity to living organisms. The study of hydrazine compounds in this laboratory began about two decades ago and developed into a large pharmacological and toxicological research program that included also environmental considerations (Back and Thomas 1970). One of the environmental pollution aspects of the program began with the evaluation of these propellants in water of different hardness (Slonim 1975). This was followed by determining polarographically the fate of hydrazine in different water sources or aquatic systems (Slonim and Gisclard 1976). Subsequently, acute toxicity studies were conducted on the common guppy (Lebistes reticulatus Peters) using four hydrazine compounds of interest, viz., hydrazine, unsymmetrical dimethylhydrazine (UDMH), Aerozine-50 and monomethylhydrazine (MMH). The results of this bioassay study, which included other hydrazine tests (e.g., effect of pre-exposure, of aged solutions, and on the DO level), were described in a previous report (Slonim 1977).

The toxicity of these propellants were evaluated next on other species of aquatic organisms such as mosquitofish (Gambusia affinis) and amphibians. Two different studies were conducted on amphibians: One utilized amphibian eggs and the other amphibian larvae; the results of the effects of the hydrazines on hatchability and development of the eggs will be reported elsewhere. The larvae of spotted and marbled salamanders (Ambystoma maculatum and A. opacum, respectively) were used primarily in numerous static bioassays to determine the acute toxicity of hydrazine, UDMH and Aerozine-50 on these organisms. The remaining larvae were used in other tests mainly to corroborate previous experimental results (e.g., to see whether toxicity is affected by organism size, aeration of test solutions, and water hardness). The results on the larvae are presented in this paper.

MATERIALS AND METHODS

The analytical equipment and methods, preparation of the hard and soft water solutions, and the bioassay conditions, containers and procedures used in this study were described in detail previously (Slonim 1973). The preparation of solutions of the different hydrazine compounds was described shortly afterwards (Slonim 1975, 1977). In all cases aqueous samples were analyzed and bioassays conducted according to standard procedures (APHA et al. 1971). The same statistical tests described previously were used here, particularly for determining the homogeneity of the sample and for assessing the significance of the difference between conditions at the 5% level, the later usually via the Student "t" test. Some of the relevant features of the methodology will be repeated below for the sake of completeness.

The hard water in this study was the supernate of raw ground water that was tapped through stainless steel pipes, collected in large plastic carboys and allowed to stand for one week for sedimentation of iron and other substances; the clear supernate was siphoned off into a large (over 50-L capacity) plastic carboy with spigot where it was stored ready for use. This water had a pH of 7.8-8.2, DO of 6.9-7.8 mg/L, hardness (EDTA) of 400-500 mg/L (as $CaCO_3$), total alkalinity of 185-232 mg/L (as $CaCO_3$), and specific conductance of 700-870 μmho/cm. It had no detectable amount of copper (<0.01 μg/ml) or iron (<0.05 μg/ml). The copper-free quality of water was important since copper is not only toxic to aquatic organisms, but causes degradation of the three hydrazine compounds used in this study (Hoover et al. 1964). The soft water was prepared just before use by making a 1:20 dilution of the hard water with glass distilled water and placing it in a 19-L capacity carboy. It had a pH of 6.3-6.9, DO of 6.9-7.8 mg/L, hardness of 20-25 mg/L, alkalinity of 16-18 mg/L, and specific conductance of 50-65 μmho/cm.

The three hydrazine compounds were liquids from Matheson, Coleman & Bell. Hydrazine [$H_2N{\cdot}NH_2$] was in anhydrous form and over 97% pure, the remainder being water. UDMH [$(CH_3)_2N{\cdot}NH_2$] was also anhydrous and at least 99% pure. Aerozine-50 was prepared by making an equal weight mixture of hydrazine and UDMH, so that 100 mg/L Aerozine-50 consisted of 50 mg/L of each of its two components. In preparing all three hydrazine solutions, corrections were made for specific gravity and purity, so that all concentrations are expressed in terms of actual weight per volume.

The salamander larvae (Ambystoma maculatum and A. opacum) were brought into the laboratory from lakes in Western Kentucky. They were placed in plastic trays, which were compartmentalized to hold one organism per cell and were divided equally between hard and soft water. They were fed white worms (Enchytrea) twice per week and maintained at a room temperature of 23.0±2°C. After adjusting to laboratory conditions for a two-three week period, the larvae were used for the bioassays.

After 2-L test solutions were placed in gallon-size (3.8-L) bioassay jars, five larvae were immediately transferred to each jar. Duplicate jars were used per test concentration and control (diluent), so that 10 larvae were tested at each concentration of a hydrazine compound as well as hard water (HW) and soft water (SW) controls. The response of the larvae to the test compound was monitored every 1-2 h around the clock. Dead larvae were removed immediately so as not to serve as a source of food for the survivors; loss of balance and other toxic symptoms were recorded when observed along with a tally of the percent of survivors each day. The median lethal concentration (LC_{50}) values were determined for each 24-h exposure period first by graphic interpolation. Second, the data from replicate bioassays were combined (following tests of homogeneity of variances) and then analyzed by the least squares method in a computer program.

At the start of the study on salamander larvae, it seemed appropriate to determine if organism size could influence hydrazine toxicity. Thus, two bioassays on hydrazine were run simultaneously, one with large size larvae [33.6(23-37)mm and 251.3(108.6-354.3)mg] and the other with relatively smaller size ones [22.3(20-26.5)mm and 92.3(72.9-114.4)mg]. In an additional hydrazine bioassay, 20 larvae were tested in aerated solutions and compared to those in the regular (unaerated) solutions to see if aeration could improve the survivability of the larvae to a toxic level of hydrazine, 10 mg/L, in both HW and SW, respectively. Lastly, since UDMH showed significantly different effects to guppies between HW and SW solutions as well as in pre-exposure studies (Slonim 1977), the effects of varying degrees of hardness on survivability to a toxic level of UDMH, 32 mg/L, were evaluated using the remaining larvae from the same batch as the others used in this study.

RESULTS AND DISCUSSION

The acute toxicity of hydrazine, UDMH and Aerozine-50 in very hard ($\geq$400 mg/L as $CaCO_3$) and soft (20-25 mg/L) water, respectively, was determined in 11 static bioassays using 870 salamander larvae. The data pertaining to the bioassays, including the concentration range tested for each compound and the graphic LC_{50} values are presented in Table 1. It is apparent from the graphic interpolation data that all three hydrazine compounds are relatively nontoxic within the first 24 hours but become more toxic as the exposure period is increased, that UDMH is the least toxic of the three compounds, and that differences between hard and soft water solutions of each compound become more pronounced at 96 h than at the shorter exposure periods.

All three bioassays on hydrazine were conducted within two weeks of each other at a temperature of 22.9(22.2-24.0)°C; the first two, in which the larvae were divided into two sizes, were conducted simultaneously. The large size larvae were only slightly more tolerant than the smaller larvae to hydrazine in either HW or SW; the largest difference between the two sizes

Table 1. General bioassay data including graphic LC_{50} values

Toxicant	No. of bio-assays	No. of larvae	Toxicant concentration range tested (mg/L)		LC_{50} by graphic interpolation (mg/L)					
			Hard water	Soft water	Hard water			Soft water		
					24	48	96	24	48	96 h
Hydrazine	3	230	10-0.32	10-0.32	>10	8.0	5.3	>10	5.2	2.3
UDMH	4	300	100-3.2	135-3.2	>100	55	26	>135	>135	108
Aerozine-50	4	340	10-0.1	10-0.1	>10	6.7	2.5	>10	>10	5.2

Table 2. 96-h LC_{50} by the least squares method; comparison between hard and soft water

Toxicant	LC_{50} with 95% confidence limits (mg/L)		Hard:Soft ratio	Significance of difference
	Hard water	Soft water		
Hydrazine	4.11 (2.78-6.09)	2.12 (1.51-2.98)	1.94	N.S.
UDMH	28.90 (16.70-50.00)	115.25 (76.61-173.39)	0.25	$p<0.05$
Aerozine-50	1.81 (0.80-4.11)	3.73 (2.51-5.54)	0.49	N.S.

appeared at 96 h but was not significant. This supports the insignificant difference in size (and larval species) reported earlier (Slonim and Ray 1975). When the replicate bioassays were combined, hydrazine in both HW and SW was more toxic with time, especially at 10 mg/L (the highest concentration tested) where the daily survival rate dropped greatly. Figure 1 shows that the computed LC_{50} of all three compounds in HW tended to decrease from 24 to 96 h, but the difference over time was not significant; all three compounds showed a similar picture in SW. The largest difference in the LC_{50} between HW and SW hydrazine solutions (4.11 mg/L and 2.12 mg/L, respectively) occurred at 96 h, suggesting that hydrazine tended to be more toxic in SW than HW; however, the difference was not significant (Table 2).

Since the DO level was reduced by 10 mg/L hydrazine to below 5 mg/L (particularly in HW between 20 to 30 h of exposure) but above the "critical level" (APHA et al. 1971), additional larvae were used to evaluate the response between aerated and unaerated HW and SW solutions of hydrazine, respectively. The result was that there was no significant difference in survivability of the larvae to 10 mg/L hydrazine between aerated and unaerated HW or SW solutions. A similar lack of significance between aerated and unaerated solutions was observed in the bioassay of UDMH in guppies (Slonim 1977).

All four bioassays on UDMH were conducted within one month of each other following the hydrazine bioassays and at a temperature of 22.6(20.5-23.5)°C. As indicated in Table 1, UDMH was nontoxic at 100 mg/L in either HW or SW during the first 24 h and also at 135 mg/L when that concentration was added in the last bioassay. Because of the high survivability at the highest concentrations tested, a reasonable estimate of the LC_{50} at 24 h could not be obtained graphically or by computer analysis; thus UDMH at 24 h is omitted in Figure 1. Like hydrazine, UDMH tended to be more toxic with increasing exposure period, but the day-to-day difference was insignificant. Unlike hydrazine, UDMH was more toxic in HW than in SW. The computed LC_{50} at 96 h was 28.9 mg/L in HW and 115.3 mg/L in SW, respectively, which is significantly different ($p<0.05$), as indicated in Table 2. This response is similar to that obtained with the guppies (Slonim 1977).

Since Henderson and Pickering (1959) reported no significant difference in UDMH between HW (300 mg/L) and SW (20 mg/L) solutions using bluegills and fathead minnows, in contrast to the results on guppies (Slonim 1977), UDMH was bioassayed at four levels of water hardness at about equal logarithmic intervals between very hard and soft water, viz., 488, 180, 65 and 24 mg/L (as $CaCO_3$). A concentration of 32 mg/L UDMH was tolerated in all four types of solutions during the first 24 h, but as the exposure period increased, survivability decreased appreciably in the very hard water but not in the three other solutions. The curve of survivability of the larvae to 32 mg/L in the four different aqueous solutions is shown in Figure 2. It is interesting to note that if one extrapolates the 300 mg/L water

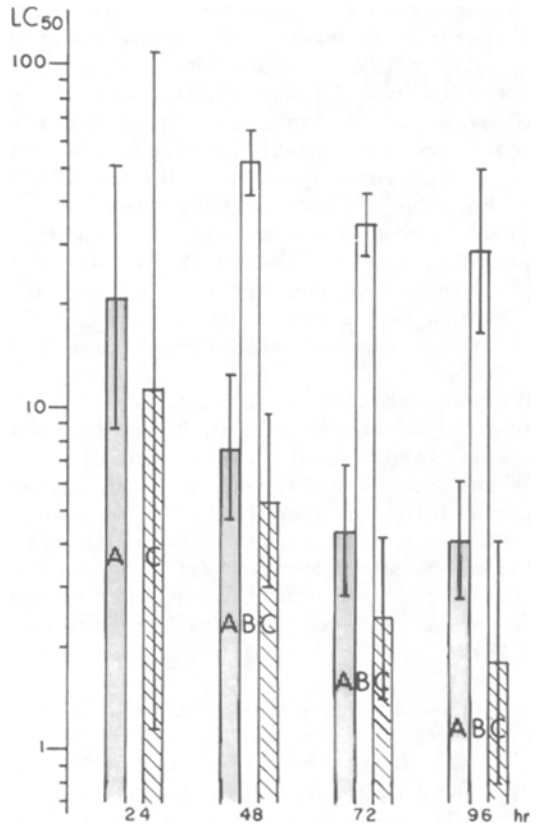

Figure 1. LC_{50} of hydrazine compounds in hard water as a function of exposure period. A = hydrazine, B = UDMH and C = Aerozine-50 (bars represent 95% confidence limits).

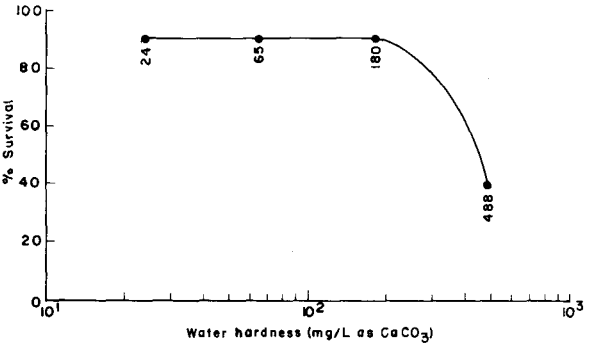

Figure 2. Survivability to 32 mg/L UDMH at 96 h versus water hardness (numbers under curve represent water hardnesses tested).

hardness used by Henderson and Pickering to this curve, the survivability of the larvae would drop from 90% to only 80%, which is insignificant, and thus their statement would fit this model as well. However, by the same analysis, survivability would drop to 50% or less as water hardness is increased to 450 mg/L or more (as $CaCO_3$). This points out once again that some of the controversy in the literature regarding the response of chemicals in HW versus SW would be minimized if the results were reported in terms of specific hardness values rather than just hard versus soft water. Some laboratories have compared results referring to water in the 140-210 mg/L range as "hard" and finding little difference in response to soft water; whereas others reported water in some parts of the world at or exceeding 500 mg/L with chemical effects changing as water becomes harder (cf., e.g., Solbé 1974).

Of the four bioassays on Aerozine-50, three were conducted within three weeks of each other and one was conducted almost a year earlier at a mean temperature of 22.1(20.0-24.0)°C; the latter bioassay was very similar to one of the other three. Aerozine-50 (Az-50), like its two components hydrazine and UDMH, became more toxic in time but not until after the first 24 h (Table 1). The computed LC_{50} also decreased slightly with increasing period of exposure in HW (Figure 1) as well as SW. Aerozine-50 appeared to be more toxic in HW than in SW after 48 h; however, the 96-h LC_{50} of 1.81 mg/L in HW and 3.73 mg/L in SW was not significant (see Table 2). Although in general Az-50 tended to be more toxic in HW than SW in three out of four bioassays (which was the opposite found for the guppies), it appeared to follow hydrazine more than its UDMH component, even to the point of showing a small but insignificant drop in the DO level (below 6 mg/L) during exposure between 20 to 30 h. The toxicity of Az-50 was in the same order of magnitude as that of hydrazine after 24 h; however, the toxicity of UDMH was significantly less than either hydrazine or Aerozine-50 ($p<0.01$).

Although there were a few aquatic studies performed on these hydrazine compounds in the past, most of them are not comparable to the present one, except the UDMH study by Henderson and Pickering cited above. The others, such as the hydrazine and UDMH study (unreplicated bioassays) on five species of fish by Heck et al. (1963) and a short (24-h) hydrazine, UDMH and Az-50 study on three fish species by Hoover et al. (1964), used synthetic reference water, which rapidly degraded hydrazine; whereas, a 72-h Az-50 study on two marine organisms by Heinemann and Rose (1966) used sea water, which caused degradation of the UDMH component of Aerozine-50. Under the same environmental conditions in this laboratory, the two species of salamander larvae were significantly more tolerant than common guppies to UDMH in both very hard and soft water ($p<0.05$). Hydrazine and Aerozine-50 were about as toxic to salamander larvae as to guppies in very hard water, but both propellants were less toxic to the larvae than to guppies in soft water ($p<0.05$).

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