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ACUTE AND BEHAVIORAL EFFECTS OF HYDRAZINE ON *LEPOMIS MACROCHIRUS*

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TECHNICAL REVIEW AND APPROVAL

AMRL-TR-78-51

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

ANTHONY A. THOMAS, MD
Director
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This study was designed to provide baseline information about the acute lethal and sublethal effects of hydrazine (H_2NNH_2) upon the bluegill, *Lepomis macrochirus*. An LC_{50} value of hydrazine with bluegills was determined as 1.08 mg/ℓ. Sublethal concentrations of hydrazine 10 to 100 times below the LC_{50} value caused the fish to become unbalanced and increase their movement. The dissolved oxygen decreased as the movement of the fish increased with increasing concentration of hydrazine. No quantification of the behavioral response was attempted; however, qualitative observations were made.

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PREFACE

This study was conducted in the Toxic Hazards Division, Environmental Quality Branch, Aerospace Medical Research Laboratory. This research was performed in support of Project 6302 "Toxic Hazards of Propellants and Materials"; Task 04, Workunit 18, from September 1977 to April 1978. Funds were provided under FY 78 Laboratory Directors Fund.

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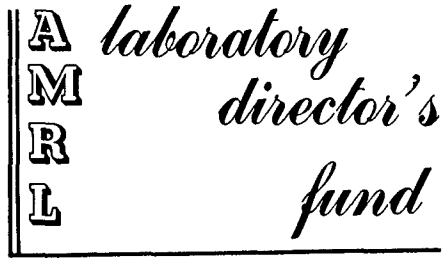

INTRODUCTION

Hydrazine, monomethylhydrazine (MMH), and unsymmetrical dimethylhydrazine (UDMH) are used as fuels in many Air Force missile systems (Lurker, 1973). Consequently, potential environmental damage may occur during manufacture, transport, and fueling operations as a result of accidental spills. Several investigators have been concerned with the effects of hydrazine in aquatic systems (Henderson et al., 1959; Heck et al., 1962; 1963; Hoover et al., 1964; Slonim, 1976, 1977; Klein et al., 1977; Greenhouse, 1976). A recent paper (Slonim, 1977) summarizes the findings of the many investigators mentioned.

The study of the bluegill, *Lepomis macrochirus*, is concerned with the effects of hydrazine pollutants entering the freshwater environment. The ultimate objective of these studies is to develop a biological monitoring system with the ability to detect pollutants by fish behavior. The system should provide the capability to assess the response or condition of aquatic organisms in hours or minutes, rather than days or weeks. The present study represents a preliminary baseline study utilizing static bioassays with bluegills exposed to various concentrations of hydrazine. This investigation will be followed by a series of continuous flow-through experiments which will emphasize behavioral response analysis.

METHODS AND MATERIALS

Two static bioassays using bluegills were conducted to derive a 96-hour LC_{50} value for hydrazine. Two 96-hour bioassays, monitoring the behavior of the fish were conducted at sublethal concentrations of hydrazine. One assay without fish, monitoring chemical parameters, was conducted.

FISH

The bluegills were obtained from Fender's Fish Farm, Baltic, Ohio. The mean weight of 20 fish was 2.3g (s = 0.919, range = 1.25 - 3.87g) and the mean length was 62.85 mm (s = 6.22, range = 51 - 74mm). A few parasites were observed. The metacercaria stage of the larval form of *Neascus* (black grub) is suspected (Ribelin et al., 1975, Fender's Fish Farm, personal communication); however, no definitive tests were run. This parasite is common in natural water systems. The fish were acclimated to water comparable to the bioassay water at least two weeks in advance of the experimental treatments. The fish were not fed two days prior to the experiments. Ten fish per concentration were used.

EQUIPMENT

The equipment consisted of five 15-gallon glass aquaria, one 15-gallon plexiglass aquarium, one Corning Model 12 pH meter, two Model 54 oxygen meters (Yellow Springs Instruments), one Coleman Junior spectrophotometer, two 50-gallon aquaria for holding tanks, and one Industrial Instruments conductivity bridge.

WATER QUALITY

The water used was a 1 : 1 dilution of tap (hard) water with in-house distilled water. The tap water was allowed to stand in plastic carboys for at least two weeks before the supernate was siphoned off. The precipitate contained iron and other substances. Table 1 depicts the preexposure water quality for the experiments.

TABLE 1

PREEXPOSURE WATER QUALITY FOR STATIC BIOASSAYS

	LC_{50} Bioassays Trials		Behavioral Bioassays Trials		Assay Without Fish
	1	2	1	2	
Hardness ($CaCO_3$, mg/ℓ)	292	240	296	248	248
Specific Conductance (μmho/cm)	446	555	450	666	460
Dissolved Oxygen (ppm)	7.1-7.3*	7.1-7.6*	7.2-7.8*	7.4-7.7*	7.1-7.4*
pH	7.2-7.3*	8.0-8.4*	7.1-7.4*	7.8+	7.2-7.4*
Water Temperature (°C)	23	23	24	20	22

*Range of Values of the Six Aquaria

+Control

HYDRAZINE

The liquid anhydrous hydrazine (H_2NNH_2) of better than 97% purity was obtained from Matheson, Coleman and Bell.[2] A stock solution was prepared by dispensing one mℓ of hydrazine from the factory glass container into a 100 mℓ volumetric flask containing 99 mℓ of glass distilled water, yielding a 10,000 mg/ℓ concentration of hydrazine.

The stock solutions for behavioral bioassay and assay without fish were made by injecting 100 microliters of hydrazine into a 100 mℓ volumetric flask containing 99.9 mℓ of glass distilled water, yielding a 1000 mg/ℓ concentration. The various concentrations required were dispensed by a microliter syringe or pipettes into 30 liters of water. The solutions were stirred for approximately three minutes to insure saturation of the solution.

The concentrations of hydrazine were measured by using a colorimetric procedure with p-dimethylaminobenzaldehyde (DMBA) as the reagent (Reynolds and Thomas, 1964). The reagent was prepared by adding four grams of DMBA to 100 mℓ of absolute ethyl alcohol; 10 mℓ of concentrated HCl was then added to the alcohol solution.

To measure concentrations of hydrazine, one mℓ of tank water was added to nine mℓ of preexposure bioassay water. One mℓ of DMBA reagent was added to this solution. After color development for 20 minutes, the optical density was read at 460 nm with a Coleman Jr. spectrophotometer. This dilution procedure allowed monitoring the concentration of hydrazine in the area of the expected LC_{50} range of 0.4 to 3.2 mg/ℓ during the experiment. Graphic interpolation of the concentration versus optical density reading was plotted to check for linearity. Then a least-squares linear regression equation was used to obtain a calibration curve. To allow for differences in experimental conditions (e.g., water quality), a separate calibration curve was prepared for each experiment. These equations were used to determine the concentration of the hydrazine throughout the 96-hour LC_{50} bioassays.

The behavioral bioassay and assay without fish concentrations of hydrazine were not monitored because the low concentrations were below the sensitivity of the procedure.

The pH, dissolved oxygen, air temperature and water temperature were recorded daily for all the experiments.

RESULTS

Table 2 shows the response (death) of the bluegills during two 96-hour LC_{50} bioassays. Ten fish per concentration were used. Using this information, Finney's Probit Analysis (Standard Methods, 1975) provided a 96-hour LC_{50} of 1.08 mg/ℓ of hydrazine. Table 3 gives the 96-hour results calculated by probit analysis.

Two separate behavioral static bioassays were conducted at sublethal concentrations of 0.01, 0.0056, 0.0032, 0.0018, and 0.0010 mg/ℓ. Ten fish per concentration were used. No quantification of the behavioral observations was attempted, but qualitative observations were made.

TABLE 2

96-HOUR LC_{50} DATA OF HYDRAZINE WITH BLUEGILLS

Concentration of Hydrazine (mg/ℓ)	Trial 1* % Death (response) per each 24-hours				Trial 2* % Death (response) per each 24-hours			
	24	48	72	96	24	48	72	96
10.0	100				**	**	**	**
5.6	100				**	**	**	**
3.2	0	90	10		90	10		
1.8	50	30	10	0	50	10	0	0
1.0	0	20	30	0	20	0	0	0
0.7	**	**	**	**	30	0	0	0
0.4	**	**	**	**	20	0	0	0
0.0	0	0	0	0	0	0	0	0

*Ten Fish Per Concentration

**Concentrations Not Tested

TABLE 3

STATISTICS FOR 96-HOUR LC_{50} PROBIT REGRESSION LINE

Intercept	-0.0820
Slope	1.0013
Std. Error of Intercept	0.1699
Std. Error of Slope	0.3444
LC_{50}	1.08 mg/ℓ
95% Confidence Limits	0.540-1.810 mg/ℓ

Behavior of the fish in one bioassay revealed that within 30 minutes after exposure to all concentrations, the fish lost balance and were swimming with their dorsal side 45° - 90° to the water surface. The fish were very active and unbalanced for approximately eight hours. The movement of the fish increased with increasing hydrazine concentration. Also, the dissolved oxygen decreased with increased fish movement. Figure 1 shows the loss of dissolved oxygen over 48 hours.

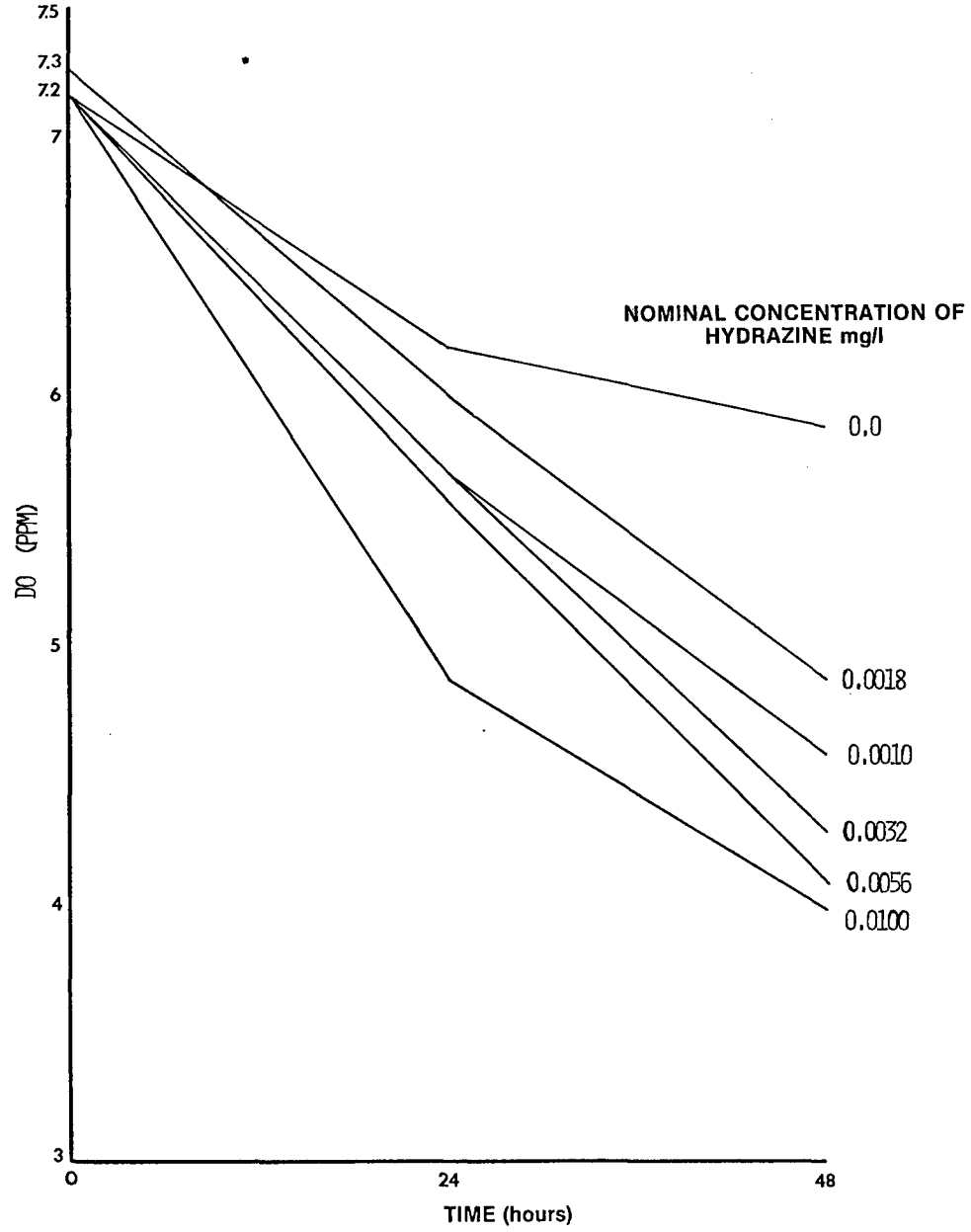

Figure 1. DO vs Time Behavioral Bioassay (Trial 1)

Using the same preparation techniques and concentrations of the pollutant, a second behavioral bioassay was done. In this case, the loss of balance and increased activity were not observed. The fish remained quiet with little movement. The dissolved oxygen decreased, but not to critical levels. To determine the effect of hydrazine alone on dissolved oxygen, an assay without fish, at concentrations the same as the behavioral bioassays was done. The resultant measurements revealed that these concentrations of hydrazine had no appreciable effect on dissolved oxygen.

The hydrazine decay curves (Figure 2) for the 96-hour LC_{50} bioassays varied between the experiments. The dissolved oxygen decreased steadily throughout the four-day experiments; however, no critical dissolved oxygen problem was encountered. The pH of the five experiments varied within one half of a pH unit during the assay without fish and the bioassays. The water temperature varied three to four degrees centigrade during each experiment.

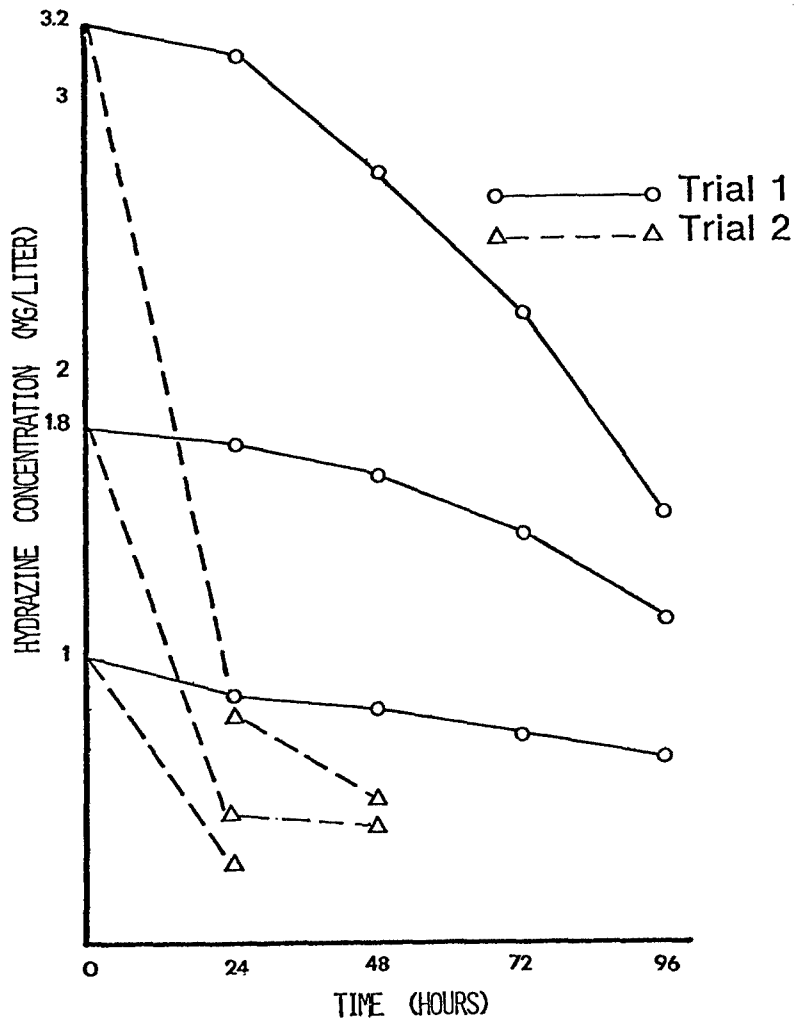

Figure 2. Hydrazine Decay Curves for LC_{50} Bioassays. The least squares regression equations for determining hydrazine concentrations are: Trial 1 ($Y = 0.1379X + 0.0408$ $r^2 = 0.999$), Trial 2 ($Y = 0.1360X + 0.0144$ $r^2 = 0.998$).

DISCUSSION

The experimental design was intended to reflect a spill situation. Static bioassay work has many shortcomings including depletion of dissolved oxygen, buildup of metabolic wastes, and general conditions not likely to be encountered in the natural lotic or lentic environment.

A 96-hour LC_{50} of 1.08 mg/ℓ with 95% confidence limits of 0.540 to 1.810 was calculated by Finney's Probit Analysis. The difference in decay curves of hydrazine during the LC_{50} bioassays may be accounted for by the pH difference (Table 4) of the test water. The water for all the experiments was from the same source, but the pH of the water during each experiment showed slight fluctuations. The common pH range of freshwater is 4.5 - 10.0 (Royce, 1972). Thus, the pH range of the bioassays could be readily encountered in the natural freshwater environment.

TABLE 4

RANGE OF pH VALUES DURING LC_{50} BIOASSAYS

	Trial 1		Trial 2	
Tank	96-Hour pH	Nominal Conc. of Hyd.	96-Hour pH	Nominal Conc. of Hyd.
1	7.3+	0.0	8.5+	0.0
2	7.4-7.6	1.0	8.0-8.6	0.4
3	7.1-7.9	1.8	8.4-8.6	0.7
4	7.4-8.0	3.2	8.4	1.0
5	7.4-7.8	5.6	8.3-8.5	1.8
6	7.6-7.9	10.0	8.3-8.6	3.2

+Initial pH of Control

This study also demonstrates the existence of reversible behavioral changes (e.g., acute increased motion and disorientation) in the bluegill at sublethal concentrations of hydrazine, providing substantiation for continued research in biological monitoring and identifying other areas of future work needing a systematic approach to advance the technology. This suggested work includes range-finding experiments for behavioral response, determination of the sensitivity of selected behavioral responses to water quality parameters, and definition of an appropriate data collection and analysis system for the selected response variables.

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