Acute Toxicity of Rodeo® and Valent X-77® To Rio Grande Silvery Minnow as Estimated By Surrogate Species: Plains Minnow, Fathead Minnow, *Hyalella azteca*, and *Chironomus tentans*

Final Report

By

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EXECUTIVE SUMMARY

Rodeo® is a postemergence herbicide for use in controlling undesirable vegetation growing in, and adjacent to, aquatic sites. Recently, the use of Rodeo® herbicide to control vegetation along irrigation canals in the Rio Grande basin has become a concern. Historic populations of the Rio Grande silvery minnow *Hybognathus amarus* were known or presumed to be present throughout most of the Rio Grande basin in New Mexico, Texas, and Mexico. Populations of Rio Grande silvery minnow have recently declined. In response to the rapid decline and threat of extinction, the United States Fish and Wildlife Service is considering listing Rio Grande silvery minnow as a federally endangered species. Because populations of Rio Grande silvery minnow may occur within irrigation canals, the United States Bureau of Reclamation initiated study of the toxicity of Rodeo® herbicide.

To estimate toxicity of Rodeo® to Rio Grande silvery minnow, we conducted 4-day renewal-acute toxicity tests with a closely related species, the plains minnow *Hybognathus placitus*, and a standard laboratory animal, the fathead minnow *Pimephales promelas*. We also conducted invertebrate 10-day solid phase sediment and water toxicity tests using bloodworms *Chironomus tentans* and amphipods *Hyalella azteca*. Fish toxicity tests were conducted by Larval Fish Laboratory, Colorado State University, Fort Collins, Colorado, and invertebrate tests were conducted by ENSR

Consulting and Engineering, Fort Collins, Colorado. The toxicants used in these studies were 1) Rodeo® (53 % glyphosate as active ingredient); 2) Valent X-77®, a non-ionic type spreader that is used in combination with Rodeo® to improve its wetting characteristics; and 3) a 3:1 mixture of Rodeo® and Valent X-77® that is the formulation currently being applied in the Rio Grande basin.

All toxicity tests followed procedures recommended by the American Society for Testing and Materials and United States Environmental Protection Agency. Dilution water was laboratory-reconstituted water prepared to match the hardness, alkalinity, and pH of the East and West Riverside Drains, New Mexico, during April through September 1988 and 1989. Nominal exposure concentrations were verified analytically.

Test results expressed as the no-observable-acute-effect concentration (NOAEC) and the median lethal concentration (LC_{50}) were:

Species	Toxicant	LC_{50}[a]	NOAEC[a]	Remarks
H. placitus	Rodeo®	>1000	1000	no significant toxic effect at 1000 mg/L
H. placitus	Valent X-77®	2.65	1.25	
H. placitus	Mix[b]	9.74	5.00	
P. promelas	Rodeo®	>1000	1000	no significant toxic effect at 1000 mg/L
P. promelas	Valent X-77®[c]	3.54	2.50	
P. promelas	Valent X-77®[c]	3.74	2.50	
P. promelas	Mix[b]	13.1	10.0	
H. azteca	Rodeo®	>1000	500	
H. azteca	Valent X-77®	25.3	12.5	
H. azteca	Mix[b]	76.8	50	
C. tentans	Rodeo®	>1000	500	
C. tentans	Valent X-77®	29.0	25	
C. tentans	Mix[b]	90.0	50	

[a]Nominal concentration, mg/L.
[b]Mix = 3:1 mixture of Rodeo® and Valent X-77®.
[c]Replicate test.

Toxicity of Rodeo® and Valent X-77® to Rio Grande silvery minnow can be inferred from the response of fathead minnows and plains minnow. Both surrogate species had approximately the same sensitivity to the toxicants. This concordance may indicate that minnows, in general, have approximately the same sensitivity to Rodeo® and Valent X-77®. In addition, plains minnow and Rio Grande silvery minnow both belong to the genus *Hybognathus* and are closely related. No exact estimate of the toxicity of Rodeo® and Valent X-77® to Rio Grande silvery minnow is possible; however, median lethal concentrations for both toxicants are probably within a factor of two of those reported for plains minnow.

PREFACE

This document reports results of toxicity tests that were conducted using surrogate species to estimate the toxicity of Rodeo® herbicide and Valent X-77® to the Rio Grande silvery minnow *Hybognathus amarus*. Chapter 1 summarizes results of 4-day renewal-acute toxicity tests conducted by the Larval Fish Laboratory, Colorado State University, Fort Collins, Colorado, using plains minnow *Hybognathus placitus* and fathead minnow *Pimephales promelas*. Chapter 2 summarizes results of invertebrate toxicity tests conducted by ENSR Consulting and Engineering, Fort Collins, Colorado. Toxicity of Rodeo® and Valent X-77® to bloodworms *Chironomus tentans* and amphipods *Hyalella azteca* was estimated using 10-day solid phase sediment and water toxicity tests. Data summaries and other support documentation are presented in appendices.

CHAPTER 1

ACUTE TOXICITY OF RODEO® AND VALENT X-77® TO RIO GRANDE SILVERY MINNOW AS ESTIMATED BY SURROGATE SPECIES: PLAINS MINNOW AND FATHEAD MINNOW

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ABSTRACT

Toxicity of Rodeo® herbicide, Valent X-77®, and a 3:1 mixture of the two materials (field formulation) to the Rio Grande silvery minnow *Hybognathus amarus* was estimated by conducting 4-day renewal-acute toxicity tests with a closely related species, the plains minnow*Hybognathus placitus*, and a standard laboratory animal, the fathead minnow*Pimephales promelas*. Four-day median lethal concentrations and 95 % confidence limits (in parentheses) for Valent X-77® and the field formulation were 2.65 (2.42, 2.91) and 9.74 (8.85, 10.7) for plains minnow; and 3.54 (2.5, 5.0) and 13.1 mg/L (10.0, 20.0) for fathead minnow. Rodeo® was not toxic to plains minnow or fathead minnow at concentrations as high as 1000 mg/L. No-observed-acute-effect concentrations (NOAEC) for plains minnow were 1000 mg/l Rodeo®, 1.25 mg/L Valent X-77®, and 5.00 mg/L field formulation. The NOAECs for fathead minnow were 1000 mg/L Rodeo®, 2.50 mg/L Valent X-77®, and 10.0 mg/L field formulation. Nominal toxicant concentrations were verified by chemical analysis. No exact estimate of the toxicity of Rodeo® and Valent X-77® to Rio Grande silvery minnow is possible; however, median lethal concentrations for both toxicants are probably within a factor of two of those reported for plains minnow.

INTRODUCTION

Rodeo® is a postemergence herbicide for use in controlling undesirable vegetation growing in, and adjacent to, aquatic sites. Toxicity of Rodeo® herbicide, and its active ingredient (glyphosate), have been thoroughly studied (Brandt 1983; Mayer and Ellersieck 1986). Recently, the use of Rodeo® along irrigation canals in the Rio Grande basin has become a concern. Historic populations of the Rio Grande silvery minnow *Hybognathus amarus* were known or presumed to be present throughout most of the Rio Grande basin in New Mexico, Texas, and Mexico. Populations of Rio Grande silvery minnow have declined and the species currently occurs in 5 % of its original range (Cochiti Reservoir downstream to Elephant Butte, New Mexico). Mechanisms responsible for the decline of Rio Grande silvery minnow include: interactions with non-native fishes; construction and operation of reservoirs; flow alterations; and declining habitat and water quality (Bestgen and Platania 1991). In response to rapid decline and threat of extinction, the United States Fish and Wildlife Service is considering listing Rio Grande silvery minnow as a federally endangered species. Because populations of Rio Grande silvery minnow may occur within irrigation canals, the U. S. Bureau of Reclamation initiated study of the toxicity of Rodeo® herbicide.

To estimate the toxicity of Rodeo® to Rio Grande silvery minnow, we conducted 4-day renewal-acute toxicity tests with a closely related species, the plains minnow *Hybognathus placitus*, and a standard laboratory animal, the fathead minnow *Pimephales promelas*, using procedures

recommended by the American Society for Testing and Materials (1990). The toxicants used in these studies were 1) Rodeo® herbicide; 2) Valent X-77®, a non-ionic type spreader that is used in combination with Rodeo® to improve its wetting characteristics; and 3) a 3:1 mixture of Rodeo® and Valent X-77® that is the formulation currently being applied in the Rio Grande basin.

METHODS AND MATERIALS

Experimental animals

Fertilized eggs of plains minnow were provided by S. P. Platania (University of New Mexico, Albuquerque). Larval fathead minnows were purchased from a commercial source. Experimental animals were acclimated to testing conditions for 48 hours before toxicity tests were initiated. Larval plains minnow and fathead minnow were approximately 48-hours old (after hatching) upon initiation of toxicity tests. Average total length and wet weight at start of the toxicity tests was 7.1 mm and 2.5 mg for plains minnow and 5.1 mm and 1.6 mg for fathead minnow.

Exposure systems

Range-finding and 4-d renewal-acute tests were conducted using 1-L glass beakers containing 0.50 liters of toxicant solutions. Contents of each beaker were renewed every 24 h. The dilution factor was 0.5. Experimental treatments (i.e., toxicant concentrations) were assigned to replicate exposure chambers using a randomized block design. Test animals were randomized to one of six treatment groups: five toxicant concentrations, and a dilution-water control. Cool-white fluorescent lamps were the only source of illumination, and a 16:8-h light:dark photoperiod was maintained.

Physical and chemical conditions

Dilution water for the toxicity tests was laboratory-reconstituted water prepared to match the hardness, alkalinity, and pH of the East and West Riverside Drains, New Mexico, during April through September 1988 and 1989 (target values: hardness, 174 mg/L as CaCO3; alkalinity, 142 mg/L as CaCO 3; and pH, 8.1). Dilution water was vigorously aerated for approximately 48 h while being heated to a test temperature of 25 ± 1 °C. Dissolved oxygen, pH, alkalinity, hardness, and specific conductance were measured daily. Water temperature was measured continuously. Dilution water characteristics for all tests had the following ranges: dissolved oxygen, 5.2-7.2 mg/L; pH, 8.0-8.3; temperature, 24.3-24.6°C; alkalinity, 111-133 mg/L as CaCO 3; hardness, 166-177 mg/L as CaCO 3; and specific conductance, 580-600 µS/cm.

Toxicant solutions

Stock solutions of Rodeo® (53.8 % isopropylamine salt of glyphosate; Monsanto Agricultural Company, St. Louis Missouri), Valent X-77® (Valent U.S.A. Corporation, Walnut Creek, California), and the 3:1 mixture of Rodeo® and Valent X-77® (henceforth called field formulation) were prepared by dissolving each toxicant in deionized water. Renewal-acute exposure concentrations were prepared by pipetting the desired amount of toxicant stock into beakers containing 0.50 L dilution water. Test solutions were stirred and transferred to exposure chambers within 30 min of preparation.

Analytical procedures

Glyphosate concentrations in each experimental treatment were measured on one occasion, at the beginning of the exposure period. Samples

were preserved by freezing at -4 °C until they could be analyzed. Upon completion of all toxicity tests, samples were submitted to Analytical Technologies, Inc., Fort Collins, Colorado for analysis as recommended by Winfield et al. (1990). Concentrations of Valent X-77® were not measured because information relevant to its formulation is protected by trademark restrictions.

Exposure conditions

Renewal-acute tests with the three toxicants were conducted simultaneously for both test species. An additional replicate test with fathead minnow and Valent X-77® was conducted to obtain an estimate of between-test variation. Fifteen larvae were placed in replicate exposure chambers. All treatments were replicated four times, with exception of the fathead minnow - Valent X-77® exposures which were replicated only two times. Obviously deformed or abnormal larvae were not selected for a test.

Larval plains minnow were not fed before or during toxicity tests because they were too small to consume brine shrimp nauplii. However, at start of toxicity tests, plains minnow had approximately one-half of their yolk remaining. Fathead minnow were offered live≤ 24-h old brine shrimp nauplii (Aquarium Products, Glen Burnie, MD) before and during toxicity tests. During a toxicity test, brine shrimp nauplii were introduced into exposure chambers approximately 1 hour before toxicant renewal. Survival of test animals was monitored at 6, 12, 24, 48, 72, and 96 hours after start of the exposure period. At conclusions of renewal-acute tests, surviving fish were killed by administering an overdose of MS-222 (Argent Chemical Laboratories, Redmond, WA) and preserved in 10 % formalin.

Statistical analysis

Median lethal concentrations were estimated using the probit or binomial methods (Stephan 1977). The median lethal concentration is defined as the concentration estimated to kill 50 % of the test animals (Rand and Petrocelli 1985). Toxicity of the field formulation was compared to that of Valent X-77® by calculating a ratio of the median lethal concentrations estimated for each toxicant. The equation had the form:

$$\frac{\text{median lethal concentration field formulation}}{\text{median lethal concentration Valent X-77®}} = \text{ratio}$$

A ratio greater than 1.0 suggested that the field formulation was more toxic than Valent X-77®; a ratio less than 1.0, that the field formulation was less toxic (Mayer and Ellersieck 1986). Ratios were based on active ingredient.

The no-observed-acute-effect concentration (NOAEC) is defined as the highest tested concentration that has no statistically significant effect on survival of the exposed population of test organisms compared to the controls. The NOAECs were estimated by conducting an analysis of variance and Dunnett's test on angular-transformed survival data. All statistical analyses were conducted using a statistical software package provided by the United States Environmental Protection Agency (Weber et al. 1989). No adjustment for mortality in the controls was made. Total number of control animals that died in any test did not exceed one individual (see Appendix A, Tables A3 and A4).

RESULTS AND DISCUSSION

Analytical procedures

Chemical analysis of glyphosate in exposure concentrations confirmed that nominal concentrations were accurate. Measured exposure concentrations were, on the average, 87 % of nominal concentrations. This value was calculated after adjustment for recovery of spiked quality-control samples. There was indication that Valent X-77® may interfere with analysis of glyphosate. In samples where Valent X-77® concentrations were greater than 2.5 mg/L, recovery of glyphosate ranged from 34 to 67 %. Since measured and nominal concentrations were in general agreement, results of toxicity tests were based on nominal concentrations.

Toxicity testing

Rodeo® was not toxic to plains minnow or fathead minnow at concentrations as high as 1000 mg/L (Table 1). However, Valent X-77® was toxic to both species. Median lethal concentrations and 95 % confidence limits (in parentheses) for plains minnow exposed to Valent X-77® were 2.65 mg/L (2.42, 2.91). Median lethal concentrations for replicated tests in which fathead minnow were exposed to Valent X-77® were 3.54 (2.5, 5.0) and 3.74 mg/L (2.5, 5.0). Median lethal concentrations for plains minnow and fathead minnow exposed to the field formulation were 9.74 (8.85, 10.7) and 13.1 mg/L (10.0, 20.0), respectively.

The ratio of median lethal concentrations of the field formulation to Valent X-77® was 0.92 and 0.90 for plains minnow and fathead minnow, respectively. Ratios between 0.5 and 1.5 are considered to be within the range of normal experimental variation (Mayer and Ellersieck 1986); therefore, no synergistic or antagonistic toxic effects were produced by the field formulation.

Because Rodeo® had no significant toxic effects over the concentration range studied, the highest concentration tested, 1000 mg/L, was designated the NOAEC for plains minnow and fathead minnow. The NOAECs for plains minnow exposed to Valent X-77® and the field formulation were 1.25 and 5.00 mg/L, respectively. The NOAECs for fathead minnow exposed to Valent X-77® and the field formulation were 2.50 and 10.0 mg/L, respectively (see Appendix A for summaries of survival, measured toxicant concentrations, and water quality).

In summary, of the three toxicants tested, Valent X-77® was most toxic to plains minnow and fathead minnow. Rodeo® showed no significant effect on survival at the studied concentrations. The field formulation was approximately 25 % less toxic than Valent X-77® by itself. This reduction of toxicity was equivalent to the proportion of Valent X-77® in the field formulation (25 %).

Toxicity of Rodeo® and Valent X-77® to Rio Grande silvery minnow can be inferred from the response of fathead minnows and plains minnow. Both surrogate species had approximately the same sensitivity to the toxicants. This concordance may indicate that minnows, in general, have approximately the same sensitivity to Rodeo® and Valent X-77®. In addition, plains

minnow and Rio Grande silvery minnow both belong to the genus *Hybognathus* and are closely related. No exact estimate of the toxicity of Rodeo® and Valent X-77® to Rio Grande silvery minnow is possible; however, median lethal concentrations for both toxicants are probably within a factor of two of those reported for plains minnow.

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Table 1. Summary of median lethal concentrations and no-observed-acute-effect concentrations for *Hybognathus placitus* and *Pimephales promelas* exposed to three toxicants.

Species	Toxicant	LC50[a]	NOAEC[a]	Remarks
H. placitus	Rodeo®	>1000	1000	no significant toxic effect at 1000 mg/L
H. placitus	Valent X-77®	2.65	1.25	
H. placitus	Mix[b]	9.74	5.00	
P. promelas	Rodeo®	>1000	1000	no significant toxic effect at 1000 mg/L
P. promelas	Valent X-77®[c]	3.54	2.50	
P. promelas	Valent X-77®[c]	3.74	2.50	
P. promelas	Mix[b]	13.1	10.0	

[a]Nominal concentration, mg/L.

[b]Mix = 3:1 mixture of Rodeo® and Valent X-77®.

[c]Replicate test.

Study Title

Toxicity of RODEO® and X-77 to

Hyalella azteca and *Chironomus tentans*

during Solid Phase Sediment and Water Toxicity Testing

Test Facility

ENSR Consulting and Engineering
Fort Collins Environmental Toxicology Laboratory
1716 Heath Parkway
Fort Collins, Colorado 80524

Test Sponsor

Colorado State University
Larval Fish Laboratory
Fort Collins, Colorado 80523

Laboratory Project ID

8505-093-117

Study Completed On

October 2, 1992

EXECUTIVE SUMMARY

Static acute toxicity tests were conducted for Colorado State University (Project Officer: Dan Beyers) to determine the acute toxicity of 1) RODEO® (53% Glyphosate as the active ingredient), 2) X-77, and 3) a mixture of the two compounds to *Hyalella azteca* and *Chironomus tentans*. Test were carried out by ENSR Consulting and Engineering at their Fort Collins Environmental Toxicology Laboratory (FCETL) using organisms obtained from in-house cultures. Project personnel were:

- Study Director(s): Kurt Drottar, Adam Cohen
- Senior Biomonitoring Technician: Stan Capps
- Study Task Manager: Denise Mikita.

Compounds were introduced into the tests by adding pre-determined quantities of chemical stock solutions to dilution water (laboratory reconstituted water prepared to match the pH, hardness, and alkalinity of water from canal systems in the Rio Grande area) poured over reference sediment (Florissant Sediment); the following nominal concentrations were tested:

(1) X-77: 0 (control), 6.25, 12.5, 25, 50, and 100 mg/L;
(2) RODEO®: 0 (control), 62.5, 125, 250, 500, and 1000 mg/L; and
(3) X-77/RODEO® Mix.: 0/0, 6.25/18.75, 12.5/37.5, 25/75, 50/150, and 100/150 mg/L.

Exposures were run for 10 days beginning on the following dates:

(1) September 2, 1992,
(2) September 8, 1992, and
(3) September 22, 1992.

Test results, expressed as the 10-day no observable acute effect concentration (NOAEC) and the 10-day median lethal concentration (LC_{50}, the concentration of the test material calculated to cause 50% mortality in the test population at the specified time of exposure) were as follows (note: concentrations are based on nominal levels for X-77 and RODEO®):

(1) H. azteca: NOAEC = 12.5 mg/L X-77; LC_{50} = 25.3 mg/L X-77 (95% C.I. = 21.7 to 29.6 mg/L)
C. tentans: NOAEC = 25 mg/L X-77; LC_{50} = 29.0 mg/L X-77 (95% C.I. = 26.1 to 32.2 mg/L)

(2) H. azteca: NOAEC = 500 mg/L RODEO®; LC_{50} = >1000 mg/L RODEO®
C. tentans: NOAEC = 500 mg/L RODEO®; LC_{50} = >1000 mg/L RODEO®

(3) H. azteca: NOAEC = 12.5/37.5 mg/L X-77/RODEO®; LC_{50} = 19.2/57.6 mg/L X-77/RODEO® (95% C.I. = 17.2 to 21.4 mg/L X-77; 51.7 to 64.1 mg/L RODEO®)

C. tentans: NOAEC = 12.5/37.5 mg/L X-77/RODEO®; LC_{50} = 22.5/67.5 mg/L X-77/RODEO® (95% C.I. = 20.2 to 25.1 mg/L X-77; 60.6 to 75.3 mg/L RODEO®)

1.0 INTRODUCTION

Static toxicity tests were conducted at ENSR Consulting and Engineering's Fort Collins Environmental Toxicology Laboratory (FCETL) to determine the acute toxicity of RODEO®, X-77, and a mixture of these chemicals to *Hyalella azteca* and *Chironomus tentans* when added to test water overlying reference, solid-phase sediment. RODEO® was introduced into the test medium as the commercial formulation (Monsanto), which contains 53 percent Glyphosate (active ingredient) by weight. Nominal test concentrations were set in terms of total RODEO® contained in each test treatment, not in terms of total glyphosate; thus, nominal glyphosate concentrations were assumed to be equivalent to 53 percent of the nominal RODEO® test concentrations established *a priori*.

The criterion for effect in these studies was death. Test results were used to calculate the 10-day no observable acute effect concentration (NOAEC) and the 10-day median lethal concentration (LC_{50}, the concentration of the test material calculated to cause 50% mortality in the test population at the specified time of exposure) for each species exposed to each compound or mixture.

All data related to this study are maintained in the FCETL archives, Data Records and Storage, 328 Link Lane #4, Fort Collins, Colorado.

2.0 MATERIALS AND METHODS

2.1 Test Materials

The test materials, Monsanto RODEO® Herbicide (RODEO®, 53 percent active ingredient by weight) and Valent X-77 Spreader, were delivered to the FCETL by Mr. Dan Beyers on 4 June, 1992, accompanied by the appropriate material safety data sheets. These materials were designated FCETL samples #3416 and #3416A, respectively.

Stock solutions were prepared prior to each chemical test by dissolving a pre-determined quantity of chemical(s), as required to yield the appropriate target chemical concentration(s), in dilution water. Stock solutions were formulated as follows:

(1) X-77: 4,000 mg/L
(2) RODEO®: 40,000 mg/L
(3) X-77/RODEO®: 4,000/12,000 mg/L

2.2 Reference Sediment

The sediment used in these studies was Florissant reference sediment, provided by Dr. Chris Ingersoll, U.S. Fish and Wildlife Service, Columbia National Fisheries Contaminant Research Center (USFWS-NFCRC). This sediment is considered "toxicologically clean", and it has been used by the USFWS-NFCRC as a negative control material for several years; a complete analytical characterization of this material is available from the USFWS-NFCRC.

2.3 Overlying Dilution Water

The overlying dilution water (FCETL RW #s 813 and 839) used in testing was a laboratory reconstituted water prepared to match the pH, hardness, and alkalinity of water from canal systems in the Rio Grande area (pH 8.1, hardness 174 mg/L as CaCO3, and alkalinity 142 mg/L as CaCO3). Initial chemical characterization of the test dilution water is presented in Table 2-1.

2.3 Test Organisms

Hyalella azteca were obtained from the FCETL in-house culture (FCETL batch #081092) and were selected for testing on the basis of size (≤3 millimeters). During testing, *Hyalella azteca* were fed a suspension of Purina Rabbit Pellets: 10 ml (4 grams), pulverized in an electric blender and mixed with 1 liter ASTM Grade I H2O.

Chironomus tentans were obtained from the FCETL in-house culture and were 10 to 14 days old at test initiation (FCETL batch #081492). During testing, *Chironomus tentans* were fed a suspension of 20 g Cerophyl combined with 2 g Tetramin Fish Flakes in 1 liter ASTM Grade I H2O.

2.4 Test Methods

Hyalella azteca and *Chironomus tentans* sediment exposure tests were conducted according to FCETL Protocol No.s 61.001 and 60.004, respectively (Appendix B), based on Nebeker et al. (1984). Testing was conducted in 500-ml beakers containing 100 ml of reference sediment and 400 ml of overlying water. After the addition of sediment to each beaker, 400 ml of dilution water, containing the appropriate amount of test material (as stock solution), was gently poured into each beaker. Nominal test concentrations were as follows:

(1)	X-77:	0 (control), 6.25, 12.5, 25, 50, and 100 mg/L
(2)	RODEO®:	0 (control), 62.5, 125, 250, 500, and 1000 mg/L
(3)	X-77/RODEO® Mix.:	0/0, 6.25/18.75, 12.5/37.5, 25/75, 50/150, and 100/150 mg/L.

Tests were initiated by placing the test organisms in the test chambers within 30 minutes of the addition of test solutions. Ten organisms were randomly distributed to each container and four replicates were tested per sediment/water treatment. Tests were conducted at 20°C under fluorescent lighting with a photoperiod of 16 hours light and 8 hours dark. An aeration pipette was set in each test chamber approximately 3 cm below the water surface to provide aeration throughout testing. Temperature, pH, and dissolved oxygen concentrations were measured daily.

Observations of mortality were made after 10 days; test organisms generally were not visible during the course of the study due to the turbidity of the test solutions. Neither test solutions nor sediments were renewed during the study. At test termination (test day 10), overlying water was decanted off, and final survival rates were determined by pouring the test sediments

into glass pans and rigorously searching the material for live and/or dead organisms. Generally, organisms that died during testing could not be found due to rapid degradation of tissues; discrepancies between initial and final organism counts were assumed to be the result of test organism mortality.

2.5 Data Analysis

NOAEC values were calculated using an IBM personal computer running TOXSTAT Version 3.3 software (Gulley et al. 1991). Survival data were first transformed by arc sine square-root and analyzed for conformance to assumptions of normality and homogeneity of variance. These assumptions were verified by Shapiro-Wilk's test and Bartlett's test, respectively ($p \leq 0.01$). If assumptions were proved valid for a given data set, one-way analysis of variance (ANOVA), followed by Dunnett's Procedure (U.S. EPA 1991) ($p \leq 0.05$), was used to compare the survival data. If assumptions were not valid, Steel's Many-One Rank Test was used to make the comparison.

LC50 values were determined using the TOXDAT Multimethod (USEPA 1985), ToxCalc version 3.4 (Tidepool Scientific 1992), and Trimmed Spearman Karber (Hamilton et al. 1977) computer programs, according to guidance provided by the USEPA (1991, 1985). For those tests in which control mortality was observed, survival among test treatment groups was corrected using Abbott's formula.

2.6 Analytical Chemistry

Water samples were collected at the initiation and termination of RODEO® testing for chemical analysis of glyphosate concentrations. Samples were turned over to Mr. Dan Beyers who delivered them to Analytical Technologies, Inc., Fort Collins, Colorado for analysis by High Pressure Liquid Chromatography. Again, water samples were analyzed for total glyphosate concentrations, which were assumed to be 53 percent of the nominal test concentrations of RODEO®. X-77 concentrations were not measured as part of the present study.

TABLE 2-1

Initial Chemical Characterization of Dilution Water in Sediment/Water Exposures

Parameter	X-77	RODEO®	X-77/RODEO®
Hardness (mg/L $CaCO_3$)	148[1]	150[1]	166[1]
Alkalinity (mg/L $CaCO_3$)	120[2]	120[2]	130[2]
Conductivity (μS/cm)	562	575	566
Ammonia (mg/L)	<0.1	<0.1	<0.1
Total Residual Chlorine (mg/L)	<0.05	<0.05	<0.05

[1] Hardness measurements for dilution waters adequately (±15%) matched the hardness of water collected from canal systems in the Rio Grande area (174 mg/L as $CaCO_3$).

[2] Alkalinity measurements for dilution waters adequately (±15%) matched the alkalinity of water collected from canal systems in the Rio Grande area (142 mg/L as $CaCO_3$).

3.0 RESULTS AND DISCUSSION

3.1 Chemical and Physical Monitoring

Concentrations of glyphosate measured at the initiation and termination of RODEO® sediment exposures are presented in Table 3-1. Initial measured glyphosate concentrations generally were quite similar to nominal levels (based on the assumption that RODEO® contains 53 percent glyphosate as the active ingredient), with only 3 out of 24 samples containing measured concentrations that were less than 85 percent of nominal. It is apparent from the disparity between initial and terminal measurements that glyphosate was not overly stable in the test medium. Final measured concentrations were significantly lower than initial values, ranging from 3 to 57 percent of nominal.

During testing, other water quality parameters generally were within acceptable limits. Among all test chambers, dissolved oxygen concentrations remained ≥ 3.7 mg/L (49 percent of saturation at 4,900 feet elevation above sea level); water temperature was maintained at 20 ± 1°C. pH values recorded in the three tests (either species) ranged as follows:

(1)	X-77:	7.2 - 8.4
(2)	RODEO®:	5.9 - 8.4
(3)	X-77/RODEO® Mix.:	6.6 - 8.4

3.2 Biological Monitoring

3.2.1 X-77 Sediment Exposure

Survival of *Chironomus tentans* was ≥90 percent among treatment groups exposed to ≤12.5 mg/L X-77; 100 percent mortality was observed among groups exposed to ≥50 mg/L X-77 (Table 3-2). Five percent mortality of control organisms was observed during the study. Based on statistical analyses (Steel's many-one rank test) of *Chironomus tentans* survival data, the NOAEC for X-77 was 25 mg/L, and the LC_{50} value was 29.0 mg/L (95% confidence interval 26.1 to 32.2 mg/L) (Table 3-3).

Hyalella azteca were slightly more sensitive to X-77 exposure during solid-phase sediment testing. Again, survival ranged from ≥90 percent among groups exposed to ≤12.5 mg/L X-77, and 100 percent mortality occurred in the 100 mg/L

treatment group (Table 3-2). Based on Steel's many-one rank test, the NOAEC for *Hyalella azteca* was 12.5 mg/L X-77, and the LC_{50} value was 25.3 mg/L (95% confidence interval 21.7 to 29.6 mg/L) (Table 3-3).

3.2.2 RODEO® Exposure

RODEO® was minimally toxic to the two test species, with ≥92 percent survival occurring among treatment groups exposed to ≤500 mg/L RODEO® (nominal). Survival among *Hyalella azteca* and *Chironomus tentans* exposed to 1000 mg/L RODEO® was 62.5 percent and 67.5 percent, respectively. The NOAEC value for both species was 500 mg/L RODEO®, and LC_{50} values for *Hyalella azteca* and *Chironomus tentans* were both greater than 1000 mg/L RODEO® (Table 3-3).

3.3.3 X-77/RODEO® Mixture Exposure

Survival rates among treatment groups exposed to the X-77/RODEO® mixture were slightly lower than those among groups exposed to X-77 alone (Table 3-2). Survival of *Chironomus tentans* and *Hyalella azteca* was ≤35 percent and ≤17.5, respectively, among treatment groups exposed to ≥25 mg/L X-77 combined with ≥150 mg/L RODEO® (nominal). Based on Steel's many-one rank test, the NOAEC value for both *Chironomus tentans* and *Hyalella azteca* was 12.5/37.5 mg/L X-77/RODEO®. LC_{50} values for the two species were 22.5/67.5 mg/L X-77/RODEO® and 19.2/57.6 mg/L X-77/RODEO®, respectively (Table 3-3).

Toxicity of the X-77/RODEO® mixture was compared to that of X-77 by calculating a ratio of the median lethal concentrations as described in Chapter 1. The ratios were 0.77 and 0.75 for *Chironomus tentans* and *Hyalella azteca*, respectively.

TABLE 3-1

Measured Glyphosate Concentrations (mg/L) in Test Solutions Containing RODEO® and a Mixture of X-77 and RODEO®

Study: *Hyalella azteca* Exposed to RODEO®

Nominal RODEO® Concentration	Nominal Glyphosate Concentration	Measured Concentration at Test Initiation (Percent of Nominal)	Measured Concentration at Test Termination (Percent of Nominal)
0 (Control)	0	<0.2	<0.2
62.5	33.1	31.0(94)	4.1(12)
125.0	66.2	63.0(95)	10.0(15)
250.0	132.5	140.0(106)	16.0(12)
500.0	265.0	270.0(102)	82.0(31)
1000.0	530.0	490.0(92)	210.0(40)

Study: *Chironomus tentans* Exposed to RODEO®

Nominal RODEO® Concentration	Nominal Glyphosate Concentration	Measured Concentration at Test Initiation (and Percent of Nominal)	Measured Concentration at Test Termination (and Percent of Nominal)
0 (Control)	0	<0.2	<0.2
62.5	33.1	32.0(97)	5.4(16)
125.0	66.2	63.0(95)	12.0(18)
250.0	132.5	140.0(106)	50.0(38)
500.0	265.0	290.0(109)	100.0(38)
1000.0	530.0	470.0(89)	300.0(57)

TABLE 3-1

Continued

Study: *Hyalella azteca* Exposed to X-77/RODEO® Mixture

Nominal RODEO® Concentration	Nominal Glyphosate Concentration	Measured Concentration at Test Initiation (and Percent of Nominal)	Measured Concentration at Test Termination (and Percent of Nominal)
0 (Control)	0	<0.2	<0.2
18.75	9.94	8.8(88)	0.35(4)
37.5	19.9	13.0(65)	1.7(8)
75.0	39.8	40.0(100)	4.5(11)
150.0	79.5	80.0(101)	11.0(14)
300.0	159.0	170.0(107)	51.0(32)

Study: *Chironomus tentans* Exposed to X-77/RODEO® Mixture

Nominal RODEO® Concentration	Nominal Glyphosate Concentration	Measured Concentration at Test Initiation (and Percent of Nominal)	Measured Concentration at Test Termination (and Percent of Nominal)
0 (Control)	0	<0.2	<0.2
18.75	9.94	8.6(86)	0.32(3)
37.5	19.9	13.0(65)	1.4(7)
75.0	39.8	18.0(45)	5.8(14)
150.0	79.5	73.0(92)	11.0(14)
300.0	159.0	150.0(94)	47.0(30)

TABLE 3-2

Mortality of Hyalella azteca and Chironomus tentans Exposed to X-77, RODEO®, and a Mixture of These Compounds in Solid-Phase Sediment Tests

Test Material	Nominal Concentration (mg/L)	Survival (%)	
		Hyalella azteca	*Chironomus tentans*
X-77	0 (Control)	100.0	95.0
	6.25	87.5	97.5
	12.5	92.5	90.0
	25	50.0[1]	75.0
	50	12.5[1]	0.0[1]
	100	0.0[1]	0.0[1]
RODEO®	0(Control)	100.0	97.5
	62.5	97.5	100.0
	125	100.0	100.0
	250	97.5	97.5
	500	92.5	100.0
	1000	62.5[1]	67.5[1]
X-77/RODEO®	0 (Control)	100.0	97.5
	6.25/18.75	100.0	95.0
	12.5/37.5	92.5	97.5
	25/75	17.5[1]	35.0[1]
	50/150	0.0[1]	0.0[1]
	100/300	7.5[1]	0.0[1]

[1] Indicates a significant difference in survival relative to that in the concurrent control group ($p \leq 0.05$).

TABLE 3-3

NOAEC and LC_{50} Values (mg/L) for Hyalella azteca and Chironomus tentans Exposed to X-77, RODEO®, and a Mixture of These Compounds in Solid-Phase Sediment Tests

	Hyalella azteca		Chironomus tentans	
Compound	NOAEC	LC_{50}	NOAEC	LC_{50}
X-77	12.5	25.3 (95% C.I.=21.7 to 29.6)	25	29.0 (95% C.I.=26.1 to 32.2)
RODEO®	500	>1000	500	>1000
X-77/RODEO® Mix.	12.5/37.5	19.2/57.6 (95% C.I.=17.2 to 21.4 mg/L X-77; 51.7 to 64.1 mg/L RODEO®)	12.5/37.5	22.5/67.5 (95% C.I.=20.2 to 25.1 mg/L X-77; 60.6 to 75.3 mg/L RODEO®)

4.0 PROTOCOL DEVIATIONS

According to the protocol (Appendix B), sediment exposures were to be conducted in 1-L beakers containing 200 ml of sediment and 800 ml of overlying water. Due to a shortage of reference sediment, however, tests were actually conducted in 500-ml beakers containing 100 ml of sediment and 400 ml of overlying water. It is unlikely that such a modification would have altered the test results. To the best of the Study Directors' knowledge, no other protocol deviations occurred during the conduct of these studies.

5.0 LITERATURE CITED

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USEPA. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. EPA/600/4-85/013.

APPENDIX A

SUMMARIES OF EXPOSURE CONDITIONS AND MEASURED RESPONSES FOR PLAINS MINNOW AND FATHEAD MINNOW EXPOSED TO RODEO®, VALENT X-77®, AND A MIXTURE OF RODEO® AND VALENT X-77®

Table A1. Water quality characteristics of dilution water in renewal-acute tests of the toxicity of three materials to *Hybognathus placitus*. Values are: mean (standard error).

	Toxicant		
	Rodeo®	Valent X-77®	Mix[a]
Dissolved oxygen (mg/L)	6.7(0.17)	6.6(0.17)	6.7(0.19)
pH	8.2(0.025)	8.2(0.025)	8.2(0.025)
Temperature (°C)	24.5(0.0250)	24.5(0.0250)	24.5(0.0250)
Alkalinity (mg/L)	128(2.50)	128(2.50)	128(2.50)
Hardness (mg/L)	168(1.93)	168(1.93)	168(1.93)
Conductivity (μS/cm)	600(0.0)	600(0.0)	600(0.0)

[a]Mix = 3:1 mixture of Rodeo® and Valent X77®.

Table A2. Water quality characteristics of dilution water in renewal-acute tests of the toxicity of three materials to *Pimephales promelas*. Values are: mean (standard error).

	Toxicant			
Toxicant:	Rodeo®	Valent X-77®[a]	Valent X-77®[a]	Mix[b]
Dissolved oxygen (mg/L)	6.1(0.46)	6.0(0.47)	6.1(0.14)	5.9(0.42)
pH	8.3(0.0)	8.3(0.00)	8.2(0.075)	8.3(0.00)
Temperature (°C)	24.5(0.00)	24.5(0.00)	24.4(0.0577)	24.5(0.00)
Alkalinity (mg/L)	123(4.13)	123(4.13)	121(2.00)	123(4.13)
Hardness (mg/L)	169(2.68)	169(2.68)	170(2.50)	169(2.68)
Conductivity (μS/cm)	590(2.5)	590(2.5)	580(2.5)	590(2.5)

[a]Replicate tests.
[b]Mix = 3:1 mixture of Rodeo® and Valent X77®.

Table A3. Summary of mortality of *Hybognathus placitus* exposed to three toxicants for 4-d exposure period. Number of replicates was four. Number of test animals per replicate was fifteen.

Nominal toxicant concentration (mg/L)	Cumulative dead per replicate			
	1 day	2 day	3 day	4 day
Rodeo®				
1000	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
control	0, 0, 0, 0	0, 0, 0, 1	0, 0, 0, 1	0, 0, 0, 1
Valent X-77®				
5.00	15, 15, 15, 15			
2.50	1, 4, 10, 4	1, 4, 10, 4	1, 4, 10, 4	1, 4, 10, 6
1.25	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 1, 1
0.625	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
0.313	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
control	0, 0, 0, 0	0, 0, 0, 1	0, 0, 0, 1	0, 0, 0, 1
Mix[a]				
15:5	15, 15, 15, 15			
7.5:2.5	13, 7, 0, 8	13, 7, 0, 8	13, 7, 0, 8	13, 7, 0, 8
3.75:1.25	1, 0, 0, 0	1, 0, 0, 0	1, 1, 0, 0	1, 1, 1, 0
1.875:0.625	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	1, 2, 1, 1
0.9375:0.3125	0, 0, 0, 0	0, 0, 0, 0	0, 1, 0, 0	0, 1, 1, 0
control	1, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0

[a]Mix = 3:1 mixture of Rodeo® and Valent X77®.

Table A4. Summary of mortality of *Pimephales promelas* exposed to three toxicants for 4-d exposure period. Number of replicates was two or four and is indicated. Number of test animals per replicate was fifteen.

Nominal toxicant concentration (mg/L)	Cumulative dead per replicate			
	1 day	2 day	3 day	4 day
Rodeo®				
1000	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
control	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
Valent X-77®, Replicate 1				
5.00	15, 12	15, 13	15, 13	15, 13
2.50	0, 0	0, 0	0, 0	0, 0
1.25	0, 0	0, 0	0, 0	0, 0
0.625	0, 0	0, 0	0, 0	1, 0
0.312	0, 0	0, 0	0, 0	0, 0
control	0, 0	0, 0	0, 0	0, 0
Valent X-77®, Replicate 2				
5.00	15, 15			
2.50	0, 0	0, 0	0, 0	0, 0
1.25	0, 0	0, 0	0, 0	0, 0
0.625	0, 0	0, 0	0, 0	0, 0
0.312	0, 0	0, 0	0, 0	0, 0
control	0, 0	0, 0	0, 0	0, 0
Mix[a]				
15:5	15, 15, 15, 15			
7.5:2.5	0, 0, 0, 0	0, 0, 0, 0	0, 0, 3, 0	0, 1, 4, 1
3.75:1.25	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
1.875:0.625	0, 0, 0, 0	0, 0, 0, 0	0, 0, 1, 0	0, 0, 1, 0
0.9375:0.3125	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
control	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0

[a]Mix = 3:1 mixture of Rodeo® and Valent X77®.

Table A5. Summary of toxicant concentrations in 4-day renewal-acute tests with plains minnow. All concentrations are mg/L.

Toxicant: Rodeo®

Nominal Rodeo® Concentration	Nominal Glyphosate Concentration	Measured Glyphosate Concentration
1000.	538	470
0.000	0.000	< 0.200

Toxicant: 3:1 mixture of Rodeo® and Valent X-77®

Nominal Rodeo® Concentration	Nominal Glyphosate Concentration	Measured Glyphosate Concentration
15.0	8.03	2.70
7.50	4.01	3.50
3.75	2.01	1.80
1.88	1.00	1.10
0.938	0.500	0.540
0.000	0.000	< 0.200

Table A6. Summary of toxicant concentrations in 4-day renewal-acute tests with fathead minnow. All concentrations are mg/L.

Toxicant: Rodeo®

Nominal Rodeo® Concentration	Nominal Glyphosate Concentration	Measured Glyphosate Concentration
1000.	538	465
0.000	0.000	< 0.200

Toxicant: 3:1 mixture of Rodeo® and Valent X-77®

Nominal Rodeo® Concentration	Nominal Glyphosate Concentration	Measured Glyphosate Concentration
15.0	8.03	5.40
7.50	4.01	3.00
3.75	2.01	1.40
1.88	1.00	1.00
0.938	0.500	0.600
0.000	0.000	< 0.200

APPENDIX B

TEST PROTOCOLS

STANDARD GUIDE FOR CONDUCTING ACUTE TOXICITY TESTS WITH FISHES

1.0 Scope and Application

1.1 Description

This method estimates the acute toxicity of a test material added to dilution water to selected fishes in a 96-h, static-renewal test.

1.2 Basis

This protocol is based on methods recommended by ASTM (1990) and USEPA (1985).

1.3 Method Summary

Test organisms will be maintained in the following treatments for the duration of the 96-h exposure period: 1) five toxicant concentration treatments; 2) one dilution-water treatment (control); and 3) if a solvent is used, one dilution water and solvent treatment (solvent control) in which the solvent concentration is equivalent to that in the toxicant treatment with highest concentration of solvent. Each treatment will have 2 replicates. Observations of effects on the organisms in each test chamber will be made periodically and analyzed to determine 96-h LC_{50}s.

2.0 Test Organisms

2.1 Fathead Minnow (*Pimephales promelas*)

Fathead minnow will be obtained from Aquatic Biosystems, Fort Collins, Colorado. At the start of a toxicity test, all fish will be less than 72-h old (post-hatch). Test organisms will not be fed before or during the exposure period.

2.2 Plains Minnow (*Hybognathus placitus*)

Plains minnow will be obtained from reproducing cultures maintained at the Larval Fish Laboratory's Wet Laboratory. Cooperators from the University of New Mexico (Steven P. Platania) will be responsible for producing the number of fish required for the toxicity tests. At the start of a toxicity test, all fish will be less than 24-h old (post-hatch). Test organisms will not be fed before or during the exposure period.

3.0 Apparatus and Test Conditions

3.1 Test Chambers

Test chambers will be 1-L glass beakers containing 0.75 L of test water.

3.2 Dilution Water

Dilution water will be supplied by ENSR Consulting and Engineering, Fort Collins, Colorado. Dilution water will be reconstituted water prepared to match the hardness, alkalinity and pH of water in the canal systems of interest.

3.3 Test Material

Test materials will be: 1) Rodeo (glyphosate); 2) X-77; and 3) a 3:1 mixture of Rodeo and X-77. Test materials will be supplied by the U.S. Bureau of Reclamation and the Middle Rio Grande Water Conservancy District (District) to ensure that the materials are the same as those used by the District in their spray programs.

3.4 Dissolved Oxygen

Dissolved oxygen concentration in test chambers will be 60-100% of saturation. Test chambers will not be aerated unless dissolved oxygen concentrations decline to less than 60% of saturation.

3.5 Test Temperature

Test temperature will be 20 ± 2°C for fathead minnow and 25 ± 2°C for plains minnow. Testing will be conducted in a temperature-controlled water bath.

3.6 Photoperiod

A 16-h light and 8-h dark photoperiod will be maintained before and during the exposure period.

4.0 Testing Procedure

4.1 Test Concentrations

Test concentrations to be used in the definitive tests will be selected based on results of range-finding tests. In range-finding tests, groups of five organisms will exposed to five widely spaced exposure concentrations and a control for 24 h. For definitive tests, test animals will be exposed to five toxicant concentrations (dilution factor of 0.5) and a control for 96 h.

4.2 Number of Test Organisms and Replicates

Fifteen animals will be randomly assigned to replicate exposure chambers. Each treatment will be replicated four times for a total of sixty organisms per test concentration.

4.3 Controls

Controls will consist of the same dilution water used in test solutions but without the addition of test material. Use of a solvent control is not anticipated.

4.4 Test Initiation and Duration

Testing will begin by placing organisms into test solutions within 30 minutes after the test material is added to the dilution water. Test solutions will be renewed every 24 h and the duration of each test will be 96 h.

4.5 Biological Data

Observations of mortality in each test chamber will be made at least daily during a definitive test.

4.6 Chemical and Physical Monitoring

Water hardness, alkalinity, and conductivity of dilution water will be measured at the beginning and end of the exposure period. Dissolved oxygen and pH will be measured daily. Temperature will be measured continuously. Test material concentrations will be verified by chemical analysis of a sample of each test solution collected at the beginning of a test. Glyphosate concentrations will be analyzed and verified by Analytical Technologies Inc., Fort Collins, Colorado. Verification of X-77 concentrations is not possible because information relevant to its formulation is protected by trademark restrictions and is not available to the public.

5.0 Acceptability of Test

5.1 A test will be considered unacceptable if mortality of test organisms in the control exceeds 10%.

6.0 Statistical Analysis

6.1 96-h LC_{50}s

A 96-h LC_{50} will be calculated for each combination of test organism and test material. Data will be analyzed using a computer program obtained from the U.S. Environmental Protection Agency (USEPA 1985).

6.2 No Observable Acute Effect Concentration (NOAEC)

The NOAECs will be calculated for each combination of test organism and test material using the SAS system for personal computers (SAS Institute Incorporated 1988).

7.0 Report

7.1 Materials Description

The record of the results of an acceptable toxicity test will include: 1) the names of investigators and locality where the tests were conducted; 2) a thorough description of the source and composition of the test material; 3) a thorough description of the source and composition of the dilution water; and 4) a thorough description of the source, species, age, and handling of test organisms.

7.2 Testing Procedure Description

The record will also contain a description of the experimental design, test conditions, method of initiating a test, and the number of test organisms in each treatment.

7.3 Summary of Observed Physical and Chemical Conditions

Results of water-quality measurements, temperature, and analyses to verify test-material concentrations will be summarized. The methods of analysis will be reported or cited.

7.4 Definitions

Endpoints used to identify toxic effects will be defined and a general summary of the observed effects will be presented.

7.5 Data Summary

The raw data and percent mortality in each treatment will be summarized in a table.

7.6 Miscellaneous

Anything unusual about the test, any deviation from these procedures, and any other relevant information will be noted in the report.

8.0 References

ASTM (American Society for Testing and Materials). 1990. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Pages 360-379 in 1990 annual book of ASTM standards, volume 11.04. American Society for Testing and Materials, Philadelphia, Pennsylvania.

SAS Institute Incorporated. 1988. SAS/STAT user's guide, release 6.03. Cary, North Carolina.

USEPA (United States Environmental Protection Agency). 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. United States Environmental Protection Agency, EPA/600/4-85/013, Cincinnati, Ohio.

9.0 Quality Assurance

9.1 Testing methods, documentation, and records will comply with quality assurance practices recommended by the U.S. Environmental Protection Agency (USEPA 1985).

ENSR Consulting and Engineering

ENSR Project No.: 8505-092
Protocol No.: 60.004
Effective: 2/21/92

Title: Solid Phase Sediment and Water Test With Glyphosate, X-77 and a Mixture Using *Chironomus tentans*.

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1.0 INTRODUCTION

1.1 Objective

To determine the acute toxicity of: 1) Rodeo (glyphosate), 2) X-77, and 3) a mixture of the two compounds to *Chironomus tentans*.

1.2 Sediment

The sediment used in testing will be Florissant soil provided by the USFWS NFCRC.

2.0 BASIS AND TEST ORGANISM

2.1 Basis

This protocol is based on the method described by Nebeker et al. (1984).

2.2 Test Organism

1. Species - *Chironomus tentans*
2. Age - *Chironomus tentans* will be 10 to 15 days old.
3. Source - Test organisms will be obtained from ENSR's in-house culture.
4. Feeding - *Chironomus tentans* will be fed a suspension of Tetramin dispersed in deionized water every other day during the test.

3.0 TEST SYSTEM

3.1 Dilution Water

The dilution water used in toxicity testing will be reconstituted water prepared to match the hardness, alkalinity and pH of water from canal systems in the Rio Grande area.

3.2 Test Temperature

Test temperature will be 20 $\pm$ 2°C. Testing will be conducted in an environmental chamber or a temperature controlled water bath.

3.3 Test Containers

Test containers will be 1-L beakers containing 200 ml of sediment and 800 ml of overlying water.

3.4 Photoperiod

The photoperiod will be 16-hours light and 8-hours dark.

3.5 Aeration

The test beakers will be aerated gently throughout the test. The aeration pipette will be approximately 3 cm below the surface of the water to avoid disturbance of the sediment and unnecessary turbidity.

4.0 TEST DESIGN

4.1 Sediment/Water Mixture

Sediment (200 ml) will be placed in each beaker. After addition of sediment, 800 ml of dilution water containing the appropriate amount of test material will be gently poured into each beaker.

4.2 Test Concentrations

The test concentrations for the single compound Rodeo and X-77 studies will be determined by range finding studies. The range finding studies will consist of three widely spaced concentrations and a control. At least five test organisms will be tested per treatment. The single compound definitive studies will consist of five concentrations and a control with a dilution factor of 0.5. The mixture study will be conducted using glyphosate and X-77 in dilution water at a ratio of 3:1. The test concentrations for the mixture study will be based on the single compound studies assuming additive toxicity.

4.3 Number of Test Organisms

Forty *Chironomus tentans* will be exposed to each treatment. Ten organisms will be randomly assigned to each test chamber and four replicates will be tested per treatment.

4.4 Controls

The controls will consist of the same dilution water/sediment mixture without the addition of the test material.

4.5 Test Initiation

Testing will be initiated by addition of the test organisms within 30 minutes of test material addition.

4.6 Chemical and Physical Monitoring

At a minimum, the following measurements will be made:

1. Hardness, alkalinity, and conductivity will be measured in the dilution water at the beginning of the test.
2. Dissolved oxygen, pH and temperature will be measured in each treatment daily.
3. Glyphosate concentrations will be measured in each treatment at test initiation and termination.

4.7 Biological Monitoring

Observations of mortality in each test chamber will be made at 10 days.

4.8 Test Duration

The test duration will be 10 days.

4.9 Calculations

Test results will be used to calculate the 10-day median lethal concentration (LC_{50}, the calculated concentration of test material which causes 50 percent mortality in the population of test organisms at the specified time of exposure) and the no observable acute effect concentration (NOAEC). The NOAEC will be calculated using Dunnett's procedure or Steel's many-one rank test (with arcsine squareroot transformation). The LC_{50} values and NOAEC will be calculated using a computer program.

4.10 Quality Criterion

The test will not be considered acceptable if control mortality exceeds 30 percent.

5.0 TEST REPORT

The report will be a typed document describing the results of the test and will be signed by the Study Director and Quality Assurance Unit. The report will include, but not be limited to the following:

- A copy of all raw data.
- Name of test, Study Director, and laboratory, and date test was begun.
- A detailed description of the test material, including its source, composition, known physical or chemical properties, and any information that appears on the sample container or has been provided by the Sponsor.
- The source of the overlying water, its chemical characteristics, and a description of any pretreatment.

- Detailed information about the test organisms, including scientific name, age, life stage, source, history, acclimation procedure, and food used.
- A description of the experimental design and the test chambers, the volume of solution in the chambers, the way the test was begun, the number of organisms per treatment, and the lighting.
- A description of any aeration performed on test solutions before or during the test.
- Definition of the criterion used to determine the effect and a summary of general observations on other effects or symptoms.
- Percentage of organisms that died or showed the effect.
- Anything unusual about the test, any deviations from the protocol, and any other relevant information.

6.0 LITERATURE CITED

Nebeker, A.V., M.A. Cairns, J.H. Gakstatter, K.W. Malueg, G.S. Schuytema and D.F. Krawczyk. 1984. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. Environ. Tox. Chem. 3:617-630.

USEPA. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. EPA/600/4-85/013.

7.0 PROCEDURAL COMPLIANCE

All test procedures, documentation, records, and reports will comply with USEPA (1985) general guidance on Good Laboratory Practices related to effluent toxicity testing. To this end, random audits of the test may be scheduled while the test is in progress. The raw data will be checked and compared to protocol requirements and Standard Operating Procedures, and the final report will be audited for accuracy and signed, if satisfactory, by both the Study Director and an individual from the Quality Assurance Unit.

8.0 PROTOCOL AMENDMENTS AND DEVIATIONS

All changes (i.e., amendments, deviations, and final report revisions) of the approved protocol plus the reasons for the changes must be documented in writing. The changes will be signed and dated by the Study Director and maintained with the protocol. All amendments must be authorized in advance by the Sponsor.

9.0 SPONSOR AND STUDY DIRECTOR APPROVAL

Sponsor Approval: Verbal Authorization Date: 9-1-92

Study Director: Adam S. Date: 9-1-92

ENSR Consulting and Engineering

ENSR Project No.: 8505-092
Protocol No.: 60.004
Effective: 2/21/92

Title: Solid Phase Sediment and Water Test With Glyphosate, X-77 and a Mixture Using *Chironomus tentans*.

Study Sponsor:
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Project Manager/Study Director: Kurt R. Drottar

1.0 INTRODUCTION

1.1 Objective

To determine the acute toxicity of: 1) Rodeo (glyphosate), 2) X-77, and 3) a mixture of the two compounds to *Chironomus tentans*.

1.2 Sediment

The sediment used in testing will be Florissant soil provided by the USFWS NFCRC.

2.0 BASIS AND TEST ORGANISM

2.1 Basis

This protocol is based on the method described by Nebeker et al. (1984).

2.2 Test Organism

1. Species - *Chironomus tentans*
2. Age - *Chironomus tentans* will be 10 to 15 days old.
3. Source - Test organisms will be obtained from ENSR's in-house culture.
4. Feeding - *Chironomus tentans* will be fed a suspension of Tetramin dispersed in deionized water every other day during the test.

3.0 TEST SYSTEM

3.1 Dilution Water

The dilution water used in toxicity testing will be reconstituted water prepared to match the hardness, alkalinity and pH of water from canal systems in the Rio Grande area.

3.2 Test Temperature

Test temperature will be 20 $\pm$ 2°C. Testing will be conducted in an environmental chamber or a temperature controlled water bath.

3.3 Test Containers

Test containers will be 1-L beakers containing 200 ml of sediment and 800 ml of overlying water.

3.4 Photoperiod

The photoperiod will be 16-hours light and 8-hours dark.

3.5 Aeration

The test beakers will be aerated gently throughout the test. The aeration pipette will be approximately 3 cm below the surface of the water to avoid disturbance of the sediment and unnecessary turbidity.

4.0 TEST DESIGN

4.1 Sediment/Water Mixture

Sediment (200 ml) will be placed in each beaker. After addition of sediment, 800 ml of dilution water containing the appropriate amount of test material will be gently poured into each beaker.

4.2 Test Concentrations

The test concentrations for the single compound Rodeo and X-77 studies will be determined by range finding studies. The range finding studies will consist of three widely spaced concentrations and a control. At least five test organisms will be tested per treatment. The single compound definitive studies will consist of five concentrations and a control with a dilution factor of 0.5. The mixture study will be conducted using glyphosate and X-77 in dilution water at a ratio of 3:1. The test concentrations for the mixture study will be based on the single compound studies assuming additive toxicity.

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Sponsor Approval: Verbal Authorization Date: 9-1-92

Study Director: Adam S. Cole Date: 9-1-92