

**ECOLOGICAL EFFECTS BRANCH
DATA EVALUATION REPORT**

1. **CHEMICAL:** SAN 582H

2. **TEST MATERIAL:** SAN 582 H technical, technical, 97% ai, Lot No. 9024, received June 25, 1991

3. **STUDY TYPE:** 96 hour static Acute Toxicity Test using *Cyprinodon variegatus*

4. **STUDY IDENTIFICATION**

Study Director: Wheat, Jeffrey

Study Laboratory: Toxicon Environmental Science, Jupiter, Fla.

Study Dates: September 3-7, 1991

Study Identification: Project No. J9106004D

Study Sponsor: Sandoz Crop Protection Corp.

EPA Identification: MRID 423366-03

5. **REVIEWED BY:**

Brian Montague, Fisheries Biologist

Ecological Effects Branch

Environmental Fate & Effects Division (H7507C)

Brian Montague
10/15/92

6. **APPROVED BY:**

Les Touart, Supervisory Biologist

Ecological Effects Branch

Environmental Fate & Effects Division

LT
10-22-92

7. **CONCLUSIONS:**

The study is acceptable for registration purposes. The LC₅₀ value of 7.2 mg/L would classify the chemical as moderately toxic to sheepshead minnow, *Cyprinodon variegatus*. The NOEL for this study is 4.27 mg/L. Slope was not calculable by binomial methods.

8. **RECOMMENDATION:**

N/A.



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9. **Submission Purpose:** Study was submitted to satisfy estuarine fish toxicity data requirements for registration of SAN 582-H active ingredient in Frontier herbicide.

10. **Study Protocol and Design:**

Methodology was based on FIFRA 40 CFR part 160 guidelines for testing of estuarine fish.

Dilution Water and Test Solutions Preparations: Dilution water was natural saltwater pumped from a shallow well, carbon filtered, and adjusted to a salinity of 20‰ by addition of aerated freshwater. Pesticide analysis showed no contaminants.

DMF solvent was used as a carrier to prepare a stock solution of 250,008 mg/L. This was diluted to 2.6, 4.3, 7.2, 12, 20 mg/L nominal concentrations. Maximum DMF concentrations was 80 ppb in the highest concentration.

Test Organisms: Test fish were obtained from Aquatic Biosystems, Fort Collins, Co. All were juveniles received approximately one month prior to test initiation. Acclimation was conducted at 22-24°C, and a salinity of 19-20 parts/thousand. Daily feedings of brine shrimp and flake food were provided up to 48 hours before testing. Average length ranged from 25-32 mm. Wet weight ranged from 0.43 - 1.0 grams (mean 0.77 grams). These were based on control fish measurements performed at test termination.

Test Materials and Study Design: Based on range testing study nominal concentrations of 2.6, 4.3, 7.2, 12, and 20.0 mg/L were employed the study. Seven 28.7 L glass aquaria containing 25L of test solution were housed in a temperature control water bath maintained at 22±1°C. Tanks were covered. A 16D/8N photoperiod at 408-525 lux intensity was employed. Ten fish were used per test vessel and were randomly distributed 30 minutes after preparation of test vessel concentrations. Loading was approximately 0.31 gm fish tissue/L (based on control fish at termination). Daily survival/mortality data was entered along with behavioral abnormalities. Temperature was monitored continuously in one test vessel and daily in other vessels. Salinity, pH and D.O. were also measured daily. Water samples were collected from all test vessels and analyzed by gas chromatography.

11. **Reported Test Results:**

Range testing produced 20% mortality at 0.1 mg/L, 0% mortality at 1.0 mg/L, 60% mortality at 10 mg/L and 100% mortality at 100 ppm. At mean measured concentrations of 2.84, 4.27, 7.77, 11.6, and 20.2 mg/L resulting 96 hour mortality was 0%, 0%, 0%, 0%, 60%, 100%, and 100% respectively. No mortality was

experienced in controls or solvent controls. The highest concentration experienced 100% mortality within 48 hours. The 11.6 mg/L concentration experienced 90% mortality after 72 hours of exposure.

Mean temperature was 23.1°C with a range of 21.2-24.8 during the 96 hour test period. Salinity ranged from 18-20 parts/thousand, dissolved O₂ dropped from 8.0 mg/L at initiation to between 3.0 and 3.7 mg/L on day 4 in all test vessels. The pH ranged from 8.4 on day 0 to between 7.9 and 8.1 on day 4. Control tank temperatures appeared to remain at about 1°C above water bath temperature.

Based on mortality levels during the study the author calculated 48 hour LC₅₀ to be 15.3 mg/L, 72 hour LC₅₀ to be 9.9 mg/L and 96 hour LC₅₀ to be 7.2 mg/L (CLS 4.3 - 11.6).

12. Study Authors's Conclusions:

"Mortality of sheepshead minnows exposed for 96 hours to SAN582H Technical was 0 percent at mean measured concentrations $\leq$ 4.27 mg/L, 60% at 7.77 mg/L and 100% at 11.6 and 20.2 mg/L.

Based upon the characteristics of the data non linear interpretation was used to calculate the 96 hour LC₅₀. The 96 hour LC₅₀ of SAN 582H technical, based upon measured concentrations was 7.2 mg/L with 95 percent confidence limits of 4.3 and 11.6 mg/L. The slope could not be calculated by binomial probability analysis. The no observed effect concentration (NOEC) was 4.27 mg/L based upon the lack of mortality and sublethal effects at this test concentration."

13. Reviewer's Discussion:

Though O_2 levels dropped below acceptable levels at the 96 hour point it is not felt that this departure from acceptable protocol affected the final results. There was 0% mortality 4 of 7 test vessels despite low O_2 levels and the mortality that did occur appeared to follow a dose response pattern. In addition 100% mortality was seen in the highest dose by day 3 when O_2 levels were still acceptable. Other water quality parameters generally appeared to remain acceptable. Concentrations of test material also appeared to remain stable.

Adequacy of Study:

Classification: Core

Rationale: Study author's conclusions are supported by reported results of the study.

Repairability: N/A

Montague SAN582H Static Acute-Sheepshead Minnow

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
20.2	10	10	100	9.765625E-02
11.6	10	10	100	9.765625E-02
7.77	10	6	60.00001	37.69531
4.27	10	0	0	9.765625E-02
2.84	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 4.27 AND 11.6 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 7.201996

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.
