ECOLOGICAL EFFECTS BRANCH
DATA EVALUATION REPORT

1. **CHEMICAL:** SAN 582H

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

3. **STUDY TYPE:** Mysid Shrimp 96 hour Acute Toxicity Test under static test conditions

4. **STUDY IDENTIFICATION**

Study Director: Wheat, Jeffrey
Study Laboratory: Toxikon Environmental Sciences, Jupiter, Fla.
Study Dates: September 6-10, 1991
Study Identification: Project No. J9106004C
Study Sponsor: Sandoz Crop Protection Corp.
EPA Identification: MRID 423366-04

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
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10/22/92

7. **CONCLUSIONS:**

Study is not completely acceptable for registration due to unexplained inconsistencies in the reported water parameters under static conditions. The LC_{50} determined by the study author (4.8 mg/L) would appear to indicate moderate toxicity to mysid shrimp. Further explanation of O_2 level fluctuation is required before full acceptance can be made.

8. **RECOMMENDATION:**

Contact laboratory for required explanation of study report data on oxygen levels. See Reviewer's Discussion.

9. Submission Purpose:

Study was submitted to support registration of SAN 582H, active ingredient of Frontier herbicide to be used on corn and soybean crops.

10. Study Protocol and Design:

Toxikon's "Acute Toxicity to the Mysid, Mysidopsis bahia, under Static Conditions" protocol is based on FIFRA Guidelines as noted in FIFR 40 CFR Part 16D.

Dilution Water and Test Solutions Preparations: Dilution water was natural saltwater pumped from a shallow well, carbon treated and then adjusted to salinity of 20°/oo filtered laboratory freshwater (source unmentioned.).

Test solutions were prepared by first preparing a stock solution of 249,994 mg/L (12.88 gms to 50 ml of Dimethyl formamide). Test concentrations were then prepared to nominal concentration levels of 1.3, 2.2, 3.6, 6.0, and 10 mg/L prepared in 3 liter batches. Highest concentration of DMF solvent was 40 ppb.

Test Organisms: Mysid shrimp < 24 hours old were obtained in house laboratory cultures maintained in a 57L glass culture aquaria. They were fed brine shrimp nauplii and maintained at 27.1 - 28.5$_0$C and 18-20°/oo salinity for 14 days prior to test initiation before selection.

Test Material and Design: Prior to definitive testing, preliminary testing was conducted at concentrations of 0.1, 1.0, 10.0, and 100 mg/L. Based on results of this test definitive nominal concentrations were selected. 2 replicates of each concentration, control, and solvent control were employed. Test vessels were 2.5L glass dishes containing 1 liter of test solution at a depth of 4.5 cm. Mysids were impartially distributed by twos until 10 organisms were contained in each test dish. The vessels were then covered and maintained in a water bath at 22±1°C. Fluorescent lighting on a 16D/8N photoperiod provided illumination at 333-442 lux intensity. Survival and behavior observations, temperature, salinity, dissolved oxygen, and pH were monitored daily. Mysids were fed 3 drops of ≤ 24 hr. old brine shrimp nauplii supplemented with fatty acids on a daily basis. Water bath temperature was continuously monitored. Water samples were collected from all vessels at initiation and termination and analyzed for SAN 582H using gas chromatography.

11. Reported Test Results:

Preliminary testing resulted in 20%, 0%, 100%, and 100% mortality at the 0.1 mg/L, 1.0 mg/L, 10.0 mg/L and 100 mg/L test concentrations (5 mysids/level).

Definitive test mortality of mysids ranged from 0% to 90% after 96 hours of exposure. No mortality occurred at 24 hours. After 48 hours 5%, 10%, 45%, and 65% mortality occurred in the 4 highest concentrations (3.52, 3.90, 5.40, 9.66 mg/L). The 3.52 mg ai/L concentration was incorrectly dosed at test initiation and is noted under protocol deviations by the study author. The 4 highest concentrations experienced 5%, 30%, 55%, and 90% mortality after 72 hours. After 96 hours exposure 0%, 0%, 0%, 20%, 40%, 65% and 90% mortality were recorded for the control, solvent control, 2.17, 3.52, 3.90, 5.40 and 9.66 mg/L measured concentrations respectively. Lethargy was the only behavioral sign noted and was observed only at the high concentration.

Measured concentrations appeared consistent between initiation and termination <10% variation. The lowest concentration was apparently misdosed and represented 300% of the intended nominal level. Other doses were within 90-99% of the nominal estimated concentration. Temperature remained stable at 20.7 to 21.9$_{o}$C. Salinity ranged from 18-20$_{0}$/oo, and oxygen levels dropped from 7.5 mg/L on Day 0 to between 5.3 to 7.1 mg/L on Day 4. Lowest values were recorded in the 2 highest treatment dosages. The pH remained between 8.4 and 8.2 for the entire study period.

12. Study Authors's Conclusions:

"Based upon the characteristics of the data, probit analysis was used to calculate the 48-, 72-, and 96-hour LC_{50}s. The 96-hour LC_{50} of SAN 582H technical based upon mean measured concentrations was 4.8 mg/L with 95 percent confidence limits of 4.2 and 5.7 mg/L. The slope of the toxicity, curet was 5.21. The no-observed-effect-concentration (NOEC) WAS 2.17 mg/L based upon a lack of mortality and sublethal effects at this test concentration.

13. Reviewer's Discussion:

The major protocol deviation noted in this study was the misdosing of the lowest test concentration. This in effect resulted in dose 1 (3.90 mg/L) level concentrations equivalent to dose 3 (3.52 mg/L) and thus it is not felt 5 test concentrations were really tested. The dose response however appears to reflect a lower mortality level for the 3.52 mg/L concentration thus indicating a 0.40 ppm difference to have had a 20% effect on mortality over the next higher concentration. The ranking of the concentrations produces a good dose response curve and does not, in this reviewers opinion, compromise the LC_{50} value which was determined. This level was between the 3.90 and 5.40 mg/L dosage levels.

Dissolved oxygen levels fell to 4.3 mg/L on day 2 then rose to 6.0 - 6.7 mg/L on day 3 (see table 6, page 23 of report). This is miraculous given the fact that no aeration was reportedly employed during the test period(see page 12 of the report) and no reported malfunction of oxygen metering equipment occurred. This must be further explained by the laboratory. The levels are just above acceptable levels of 40%.

Adequacy of Study:

Category: Supplemental

Rationale: Report appears to contain inaccuracies. O_2 levels rose from day 1 and 2 despite the reported lack of aeration methods. This may have affected mortality through stress on the organisms.

Repairability: Yes, if the laboratory can offer a satisfactory explanation of the drop and rise in oxygen levels.

Brian Montague SAN582H Mysid Static Tox Test

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
9.66	20	18	90	2.012253E-02
5.4	20	11	55	41.19014
3.9	20	6	30	5.765915
3.52	20	1	5	2.002716E-03
2.17	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 3.52 AND 9.66 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 5.066102

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1219604	5.217897	4.676902	5.999828

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1186952	1	.3669067

SLOPE = 5.884543
95 PERCENT CONFIDENCE LIMITS = 3.85719 AND 7.911896

LC50 = 5.404067
95 PERCENT CONFIDENCE LIMITS = 4.768751 AND 6.338928

LC10 = 3.287767
95 PERCENT CONFIDENCE LIMITS = 2.570753 AND 3.807037

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