

US EPA ARCHIVE DOCUMENT

**ECOLOGICAL EFFECTS BRANCH
DATA EVALUATION REPORT**

1. **CHEMICAL:** SAN 582H, technical
2. **TEST MATERIAL:** SAN 582 H technical, 97% purity, Lot 9024
3. **STUDY TYPE:** 96 hour study examining acute toxicity to shell growth of *Crassostrea virginica* under flow through conditions.

4. **STUDY IDENTIFICATION**

Study Director: Wheat, Jeffrey
Study Laboratory: Toxicon Environmental Science, Jupiter, Fla.
Study Dates: October 25-29, 1991
Study Identification: Project No. J9106004C
Study Sponsor: Sandoz Crop protection
EPA Identification: #423366-02

5. **REVIEWED BY:**

Brian Montague, Fisheries Biologist
Ecological Effects Branch
Environmental Fate & Effects Division

Brian Montague
10/15/92

6. **APPROVED BY:**

Les Touart, Supervisory Biologist
Ecological Effects Branch
Environmental Fate & Effects Division

L. Touart
10/22/92

7. **CONCLUSIONS:**

The test was conducted with sound methodology and is acceptable for registration purposes. The EC₅₀ for shell growth reduction was 5.0 mg/L of SAN 582 H. The NOEL was ≥ 1.13 mg ai/L. The slope of the toxicity curve is 3.4.

8. **RECOMMENDATION:**

N/A.



9. Submission Purpose:

Submitted to support registration of SAN 582H, active ingredient for a new herbicide, Frontier, proposed by Sandoz Crop Protection Corporation.

10. Study Protocol and Design:

Methods and protocol are based on EPA Standard Evaluation Procedures and FIFRA 40 CFR Part 16D guidelines.

Dilution Water and Test Solutions Preparations: The dilution water was natural unfiltered saltwater from the Jupiter River Inlet in Jupiter, Fla. The seawater had an average pH of 8.0 and a salinity range of 29-33 parts/thousand. Stock solutions were prepared by addition of 4.78, 7.62, 13.25, 22.09, and 36.82 grams of chemical to 250 ml of dimethyl formamide, thus producing stocks of 18,546, 29,566, 51,410, 85,709, and 142,862 mg ai/L. These were diluted to nominal concentrations of 1.3, 2.1, 3.6, 6.1, and 10.2 mg ai/L by the diluter system.

Test Organisms: Eastern oysters, *Crassostrea virginica*, were purchased from Aquacultural Research Corporation in Dennis, Mass. and received on October 23, 2 days before test initiation. They were subjected to a 48 hour acclimation following removal of 2-5 mm of peripheral shell growth. Control oysters were measured at test termination. Umbo to distal length range was 22.0 to 35.9 mm. Wet tissue wt. ranged from 0.15 to 0.90 gms.

Test Material and Design: Test systems were composed of a glass head box which provided flow of 394 ml/minute/test chamber. This was equivalent to 1.2L of dilution water/oyster/hour. A Harvard syringe pump provided appropriate injections of test solution to each mixing chamber where magnetic stirrers blended solution with incoming dilution water. Solutions were then delivered via glass tubing to 28.7 glass aquaria maintained at an 18.7L volume by 13 cm standpipes. Thirty volume additions/24 hours were introduced. All test containers were positioned in a 23.0±1°C water bath. Twenty oysters were impartially distributed to each test chamber. Loading was 0.014 gm oyster tissue/L of test water. Temperature was logged hourly in one control vessel. Daily measurement of temperature, pH, and D.O. were performed. No supplemental feeding was performed. Test vessels were not aerated. During the study light intensity was maintained at 433-542 lux with a 16D/8N photoperiod. No replicates were employed. Water samples were collected from high, medium, and low test solutions for measurement of concentration levels using gas chromatography. Samples were removed at test initiation and termination.

11. Reported Test Results:

Mean measured concentrations of SAN 582-H were 1.3, 2.1, 3.6, 6.1, and 10.2 mg ai/L with little variance. This was equivalent to between 87 and 104% of nominal estimated concentration levels. Significant reduction was seen in mean shell growth of the highest 3 concentrations which were 3.345 mm, 3.355 mm, 3.63 mm, 3.06 mm, 2.18 mm, 1.64 mm, and 0.69 mm for the controls, solvent controls, 1.13, 2.18, 3.35, 5.26; and 9.85 mg/L concentrations, respectively. There was no mortality in any level. For statistical analysis controls were pooled. Water quality parameters remained at acceptable levels throughout the study period. Constant temperature range readings for the control vessel appeared to vary by 2°c from readings taken in the water bath. This may have been due to calibration of different monitoring equipment. Salinity remained at 29-33 pp thousand. Oxygen levels ranged from 6.1 - 7.0 mg/L with the exception of one daily reading of 5.3 mg/L on the control vessel at initiation. Readings of 6.9 - 7.0 are equivalent to 100% saturation at this salinity and temperature. The pH values ranged from 7.8 to 8.0, normal for seawater.

12. Study Authors's Conclusions:

Based on shell growth data the 96 hour EC_{50} of SAN 582H Technical to the eastern oyster, *Crassostrea virginica*, as calculated by probit analysis was 5.0 mg ai/L with 95 percent confidence limits of 4.6 and 5.6 mg ai/L. The no observed effect concentration (NOEC) was 2.18 mg ai/L. The slope of the EC_{50} line was 3.4.

13. Reviewer's Discussion:

The test appeared to have been conducted soundly and a good dose response for shell reduction has been achieved. The study author has explained that the shorter than normal initial acclimation period appeared to offer improved shell growth performance over oysters held for 4 days prior to dosing. This would not have altered the results in the opinion of the reviewer. The chemical appears to demonstrate moderate toxicity to oyster shell growth and the calculated EC_{50} of 5.0 mg/L is supported by independent agency analysis. The LOEL is 2.18 mg ai/L based on 8.7% reduction at this concentration. The NOEL is ≥ 1.13 mg/L based on lack of any significant shell reduction at this dosage. The study author felt the reduction at 2.18 mg ai/L to be insignificant. The reviewer does not agree with this as the 8% reduction follows a dose response pattern with the other treatment levels.

Adequacy of Study:

Classification: Core

Rationale: Conclusion of the study author are supported by the study results

Repairability: N/A

Montague SAN582H Shell Reduction Eastern Oyster

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
9.850001		20	17	85
.1288414				
5.26	20	10	50	58.80985
3.35	20	7	35	13.1588
2.18	20	2	10	2.012253E-02
1.13	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 2.18 AND 9.850001 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 5.26

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1564043	4.833762	3.88713	6.349311

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
4	.1243839	1
GOODNESS OF FIT PROBABILITY		
.823725		

SLOPE = 3.520581
 95 PERCENT CONFIDENCE LIMITS = 2.278939 AND 4.762223

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