

AR226 - 3257

TRADE SECRET***Study Title***

H-25434: Static, Acute, 96-Hour Screening Test to
Pimephales promelas

Laboratory Project ID: DuPont-11408

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PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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WORK REQUEST NUMBER: [REDACTED]

STUDY CODE NUMBER: [REDACTED]

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Issued by Study Director:

Laurie D. Bouchelle

Laurie D. Bouchelle
Associate Scientist

2 October 2002

Date

STUDY INFORMATION

Substance Tested: [REDACTED]

Synonyms/Codes: • H-25434

Haskell Number: 25434

Composition: [REDACTED]

Known Impurities: [REDACTED]

Physical Characteristics: Off-white, milky semi-opaque aqueous dispersion

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: September 23, 2002 / (see report cover page)

In-Life Initiated/Completed: September 23, 2002 / September 27, 2002

SUMMARY

The acute toxicity of H-25434 to the fathead minnow, *Pimephales promelas* was determined in an unaerated, 96-hour, static test.

The study was conducted with 4 concentrations of H-25434 and a dilution water control at a mean temperature of 22.9°C (range of 22.9-23.0°C). One test chamber was used per test concentration with 10 test organisms in each chamber. Based on visual observations, the water control and the 1.0 and 10 mg/L test concentrations were clear with no color at test start. The 100 mg/L test concentration was clear with an oily surface film at test start, and the 1000 mg/L test concentration was cloudy with an oily surface film at test start. All water quality parameters were within acceptable limits during the exposure.

Exposure of rainbow trout to the water control and nominal H-25434 concentrations of 1, 10, 100, and 1000 mg/L resulted in 0, 0, 0, 20, and 100% mortality, respectively, at the end of 96 hours. No mortality or sublethal effects were seen in the water control test organisms. The highest nominal concentration causing no mortality at test end was 10 mg/L. The lowest nominal concentration causing 100% mortality at test end was 1000 mg/L. Nominal H-25434 concentrations were used for calculation of LC₅₀ values.

The results are summarized as follows:

Nominal concentrations of H-25434, mg/L	dilution water control, 1, 10, 100, and 1000
96-hour LC ₅₀ (95% fiducial limits) for H-25434, based on nominal concentrations, mg/L ^a	181 (62 to 448)

H-25434 exhibited low concern for aquatic hazard^b in an unaerated, 96-hour, static, acute test using the fathead minnow, *Pimephales promelas*.

^a Peltier, W.H. and Weber, C.I., Eds. (1985). *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms*. EPA/600/4-85-013. United States Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

^b Smrcek, J., Clements, R., Morcock, R., and Rabert, W. (1993). Assessing ecological hazard under TSCA: methods and evaluation of data. *Environmental Toxicology and Risk Assessment, ASTM STP 1179*. (W.G. Landis, J.S. Hughes, and M.A. Lewis, Eds.), pp 22-39. American Society for Testing and Materials, Philadelphia.

MATERIALS AND METHODS

A. Test Solution Preparation

Test substance solutions were prepared by direct addition of H-25434 to dilution water. The nominal concentration of test substance solutions were not adjusted for purity. Test solutions were prepared by adding the appropriate amount of test substance to each test chamber which contained 15 L of Haskell Laboratory well water and stirring for approximately 30 minutes. The water control and the 1.0 and 10 mg/L test concentrations were clear with no color at test start. The 100 mg/L test concentration was clear with an oily surface film at test start, and the 1000 mg/L test concentration was cloudy with an oily surface film at test start.

B. Dilution Water

Dilution water originated from the Haskell Laboratory well which is 378-feet deep and is cased and sealed into bedrock. The hardness of the well water is adjusted to approximately 100-140 mg/L as CaCO_3 by the flow-proportioned addition of CaCl_2 . The well water is then aerated, passed through a green sand filter to remove iron, and filtered through 50-, 10-, and 0.45- μm filters to remove particulates. The water is heated or chilled as appropriate and distributed through aged polyvinyl chloride piping. The dilution water is analyzed twice yearly for major anions and cations, metals, total organochlorine and organophosphate pesticides, and polychlorinated biphenyls. The dilution water meets OECD⁽¹⁾ and ASTM⁽²⁾ criteria and specifications.

C. Test Organism Culture

Fathead minnow, *Pimephales promelas*, were reared at Haskell Laboratory. The fish were held in a 40-L, rectangular, glass holding tank (50-cm length, 25-cm width, 31-cm depth) for 101 days in continuously-flowing well water. During the 7-day period preceding the test, water temperatures ranged from 21.9 to 22.1°C (mean 22.0°C). The fish were fed freshly-hatched, live brine shrimp (San Francisco Bay Brand, Inc.)/frozen brine shrimp (San Francisco Bay Brand, Inc.)/AquaMax[®] Starter Fingerling 300 5D03 (PMI[®] Nutrition International, LLC) twice daily on weekdays and once daily on weekends and holidays. Sickness, injury, and visible abnormalities were not present in fish in the holding tank nor in the fish used for testing. Less than 0.5% mortality was seen during the 48 hours prior to use for testing. Fish were identified by labels on the holding tanks and test chambers.

D. Test Methods

Four nominal test concentrations and a dilution water control were used in this study. The nominal test concentrations were 1, 10, 100, and 1000 mg/L H-25434.

Test chambers were stainless steel aquaria [30 (length) x 30 (width) x 30 (height) cm] which held approximately 15 L of test solution (26-L maximum volume; 17.5-cm test solution depth). One test chamber was used per test solution (total of 5 test chambers; 10 fish in each test chamber). Each chamber was covered with a glass plate to prevent the fish from escaping.

Addition of fish to the test solutions was initiated after mixing of the test solutions was completed. Mortality and behavioral observations were made every 24 hours and at test end. Dead fish were removed from the test chambers during daily observations. Criteria for death were the absence of opercular movement and lack of reaction to gentle prodding. At test conclusion, all fish were humanely sacrificed using MS222 (3-aminobenzoic acid ethyl ester).

A recirculating water bath was used to maintain mean temperature in the test chambers during the 96-hour test at approximately 22.9°C with a range of 22.9 to 23.0°C. A photoperiod of 16 hours light (approximately 182 to 442 Lux) and 8 hours darkness was employed which included 30 minutes of transitional light (25 to 211 Lux) preceding and following the 16-hour light interval.

Dissolved oxygen concentration, pH, and temperature were measured in all test chambers. These measurements were taken before fish were added at test start, every 24 hours thereafter, and at test end. Test solutions were not aerated during the test and were disposed of in an appropriate manner at test end.

REFERENCES

1. Organisation for Economic Co-Operation and Development (OECD). Guideline for Testing Chemicals: 203, 17 July 1992.
2. American Society for Testing and Materials (ASTM). (1988). Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E 729-88a. Annual Book of ASTM Standards, Vol. 11.04.

TABLE 1

DISSOLVED OXYGEN AND pH OF TEST AND CONTROL SOLUTION SAMPLES

Nominal H-25434 Concentration (mg/L)	Test Initiation (0 hours)		Test Completion (96 hours)	
	Dissolved Oxygen (mg/L)	pH	Dissolved Oxygen (mg/L)	pH
Water Control	8.5	7.7	6.4	7.5
1.0	8.4	7.6	7.2	7.7
10	8.4	7.5	7.1	7.6
100	8.4	7.4	6.2	7.5
1000	8.4	7.3	7.5 ^a	7.3 ^a

a Measurement performed at 24 hours due to total mortality.

TABLE 2

MORTALITY OF *Pimephales promelas* IN AN UNAERATED, STATIC, ACUTE, 96-HOUR
TEST WITH H-25434

Nominal H-25434 Concentration (mg/L)	Number of Dead Test Organisms / Number of Test Organisms at Study Start			
	24 Hours	48 Hours	72 Hours	96 Hours
Water Control	0/10	0/10	0/10	0/10
1.0	0/10	0/10	0/10	0/10
10	0/10	0/10	0/10	0/10
100	0/10	0/10	1/10	2/10
1000	10/10	10/10	10/10	10/10