DATA EVALUATION RECORD

1. **CHEMICAL:** Oxythioquinox.
Shaughnessey No. Not Available.

2. **TEST MATERIAL:** Morestan® Technical (6-Methyl-1,3-dithiolo[4,5-b]quinoxalin-2-one; Batch No. 9-00-5012; CAS No. 2439-01-2; 95.0% active ingredient; a yellow-colored powder.

3. **STUDY TYPE:** Estuarine Fish Flow-Through Acute Toxicity Test. Species Tested: Sheepshead Minnow (Cyprinodon variegatus).

4. **CITATION:** Ward, G.S. 1990. Acute Toxicity to Sheepshead Minnow, Cyprinodon variegatus, Under Flow-Through Conditions. Laboratory ID No. J9004007b. Mobay Report No. 100226. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by Mobay Corporation, Kansas City, MO. EPA MRID No. 416075-10.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
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Signature: Louis M. Rifici
Date: 7/8/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
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Date: 7/8/91

Henry T. Craven, M.S.
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John Noles 3/3/92

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a flow-through acute saltwater fish toxicity study. The 96-hour LC_{50} of 129 µg a.i./L (based on mean measured concentrations) classifies Morestan Technical as highly toxic to sheepshead minnows. The NOEC was determined as 53 µg a.i./L, based on the lack of mortality and sublethal effects.

8. **RECOMMENDATIONS:** N/A

6 hrs

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Young sheepshead minnows (Cyprinodon variegatus) were obtained from a commercial supplier in Fort Collins, CO, and held in the laboratory, in natural seawater, for over 4 months prior to testing. During the 7 days immediately prior to test initiation, the fish were maintained in water with a temperature of 21.6°-25.2°C and a salinity of 20-22 parts per thousand (ppt). During holding, the fish were fed commercial flake and pelletted food daily. Feeding was discontinued 72 hours prior to test initiation. No mortality occurred during the 48-hour period immediately before the test.

The average length (±SD) of the test fish was 30 (22-36) mm and the range in weights was 0.34 to 1.88 g.

B. **Test System:** The test system was a proportional vacuum-siphon diluter. The test chambers were 24-L glass tanks fitted with glass automatic siphons. The test solution depth was 13 cm; the solution volume was approximately 15 L. The diluter delivered 1400 mL of test solution to each vessel per cycle (3.6 cycles per hour) for a total of approximately 8 volume replacements per day. The test chambers were randomly positioned in a temperature-controlled water bath under a 16-hour light/8-hour dark photoperiod with 30-minute dawn and dusk simulations.

Natural filtered (20 µm) seawater adjusted to a salinity of 20 ppt was used as test dilution water. The water was vigorously aerated prior to use.

A stock solution was prepared by adding 2.6317 g of Morestan Technical to 500 mL of dimethylformamide (DMF). The stock solution was pumped into the diluter mixing chamber providing a high nominal test concentration of 500 µg a.i./L. The mixing chamber solution was serially diluted to provide the lower test concentrations.

C. **Dosage:** Ninety-six-hour flow-through test. Based on the results of preliminary tests, five nominal

concentrations (65, 110, 180, 300, and 500 µg a.i./L), a dilution water control, and a solvent control (0.1 mL DMF/L) were used.

D. **Design:** Sheepshead minnows were impartially added, by two's, to the test chambers for a total of 20 fish per chamber. One chamber was used per treatment. The instantaneous loading was 0.18 g/L/day.

Observations of mortality and sublethal responses were made every 24 hours. Dead fish were removed at each observation period. The temperature, salinity, dissolved oxygen (D.O.), and pH of the test solutions were measured in each chamber at the beginning of the test and at each 24-hour observation. The temperature was also monitored hourly in the control chamber.

Morestan concentrations were measured by gas chromatography from samples taken at test initiation and termination.

E. **Statistics:** The median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) for each 24-hour interval were calculated using a computer program developed by Wheat (1989).

12. **REPORTED RESULTS:** Throughout the exposure, undissolved test material was observed on the surface of the mixing chamber. The mean measured concentrations were 34, 53, 86, 240, and 274 µg a.i./L, and each averaged approximately 50% of nominal except at the 240 µg a.i./L (mean measured) test level (Table 1, attached). Measured concentrations were fairly consistent between sampling days except at the highest two test concentrations.

The responses of sheepshead minnows are given in Table 2 (attached). The 96-hour LC_{50}, based on mean measured concentrations, was 119 µg a.i./L (95% C.I. = 90-177 µg a.i./L). The slope of the toxicity curve was 8.0. Sublethal and lethal effects were observed at concentrations >53 µg a.i./L, therefore, the no-observed-effect concentration (NOEC) was given as 53 µg a.i./L. One fish in the 34 µg a.i./L chamber was missing on day 4 and may have escaped through the siphon tube.

Dissolved oxygen ranged from 5.5 to 7.7 mg/L or 72 to 101% of saturation at 22°C and 20 ppt. The pH values ranged from 8.6 to 8.7. The temperature, as recorded by a data logger, was 20.5°-23.0°C. The salinity was 20-21 ppt.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
The author presented no conclusions.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated as follows:

The salinity of the dilution water in the study was 20-21 ppt with a pH of 8.6-8.7. The recommended salinity and pH for euryhaline fish species are 10-17 ppt and 7.7-8.0, respectively.

The temperature during the test was 20.5°-23.0°C. The recommended test temperature for sheepshead minnows is 22°±1°C.

B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the 96-hour LC_{50} value as 129 µg a.i./L (95% C.I. = 86-240 µg a.i./L) by the binomial method (see attached printout). The author's value (119 µg a.i./L) is lower and since it will better protect this species, it will be taken as the true value.

C. Discussion/Results: One fish was missing from the test chambers at 34 µg a.i./L on the last day of the test. The reviewer agrees with the author's suggestion that this fish probably escaped through the automatic siphon.

This study is scientifically sound and meets the guideline requirements for a flow-through acute saltwater fish toxicity study. The 96-hour LC_{50} of 129 µg a.i./L (based on mean measured concentrations) classifies Morestan as highly toxic to sheepshead minnows. The NOEC was determined as 53 µg a.i./L based on the lack of mortality and sublethal effects.

D. Adequacy of the Study:

(1) Classification: Core

(2) **Rationale:** N/A

(3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 06-04-91.

Table 1. Measured Concentrations of Morestan Technical During a 96-Hour Exposure of Sheepshead Minnow, Cyprinodon variegatus, Under Flow-Through Conditions

Nominal Concentration (µg/L; ppb)	*Measured Concentration (µg/L) 0 Hour	96 Hour	Mean (±SD)	Percent of Nominal
Control	ND	ND	---------	---
Sol. Ctl.	ND	ND	---------	---
65	35	32	34 (2)	52
110	55	51	53 (3)	48
180	87	84	86 (2)	48
300	270	211	240 (42)	80
500	300	248	274 (37)	55
SPIKE RECOVERY DATA				
MS	570	517	544 (37)	109

SD = Standard Deviation.

ND = Not detected; the limit of detection for the method was 1 µg/L.

MS = Matrix spike. The matrix spike consisted of test substance in dilution water. One spike concentration was analyzed, 500 µg/L.

*On Day -1, Morestan concentrations in 65, 110, 180, 300 and 500 µg/L were 45, 55, 94, 190 and 170 µg/L, respectively.

Table 2. Mortality of Sheepshead Minnow, Cyprinodon variegatus, Exposed to Morestan Technical under Flow-Through Test Conditions

Mean Measured Concentration (µg/L; ppb)	Cumulative Number Dead (Percent Mortality) 24 Hour	48 Hour	72 Hour	96 Hour
Control	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)
34	0 (0)	0 (0)	0 (0)	1 (5)
53	0 (0)	0 (0)	0 (0)	0 (0)
86	0 (0)	0 (0)	2 (10)	2 (10)[c]
240	0 (0)[a]	6 (30)[c]	14 (70)[d]	20 (100)
274	6 (30)[b]	20 (100)	20 (100)	20 (100)

[a] Three fish were dark and two exhibited a partial loss of equilibrium.

[b] Ten fish exhibited a complete loss of equilibrium and all fish were lethargic.

[c] All fish were lethargic and five exhibited a complete loss of equilibrium.

[d] All fish were lethargic and four fish exhibited a complete loss of equilibrium.

[e] Four fish were dark as compared to the color of the control fish.

LOUIS M. RIFICI OXYTHIOQUINOX CYPRINIDON VARIEGATUS 6-4-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
274	20	20	100	9.536742E-05
240	20	20	100	9.536742E-05
86	20	2	10	2.012253E-02
53	20	0	0	9.536742E-05
34	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 86 AND 240 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 128.5409

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.

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