DATA EVALUATION RECORD
FRESHWATER FISH LC_{50} TEST
GUIDELINE 72-1

1. CHEMICAL: Spinosed (also known as Factor A and Factor D)

   Shaughnessey #: 110003

2. TEST MATERIAL: XDE-105; Lot ACD13651; 88% potency as combined compounds 232105 (Factor A) and 275043 (Factor D); light grey to white solid

3. CITATION J. L. Newsted and D. E. Brock 1992. The Toxicity of XDE-105 to Bluegill (Lepomis macrochirus) in a Static Test System; Laboratory Project ID F00491; Lilly Research Laboratories, Greenfield, IN 46140; Submitted by DowElanco, Indianapolis, IN 46258-1189; MRID 43414534

4. REVIEWED BY:

   Joanne S. Edwards
   Entomologist
   Ecological Effects Branch
   Environmental Fate and
   Effects Division (7507C)

   Signature: Joanne S Edwards
   Date: 3/14/95

5. APPROVED BY:

   Leslie W. Touart
   Section Head
   Ecological Effects Branch
   Environmental Fate and
   Effects Division (7507C)

   Signature: LWT
   Date: 3/24/95

6. CONCLUSIONS: This study is scientifically sound and satisfies the guideline requirement (Gdln 72-1) for a 96-hour static acute toxicity test with the bluegill. Based upon mean measured concentrations, the 96-hour LC_{50} of XDE-105 is 5.94 mg a.i/l. This classifies XDE-105 as moderately toxic to the bluegill. The NOEC is 2.10 mg a.i/l.

7. ADEQUACY OF THE STUDY: Core

8. RATIONAL FOR CLASSIFICATION: N/A

9. BACKGROUND: New chemical EUP.

10. MATERIALS AND METHODS:

A. Test Organisms:

Guideline Criteria	Reported Information
Species Preferred species is the bluegill (*Lepomis macrochirus*)	species tested was the bluegill (*Lepomis macrochirus*)
Mean Weight 0.5-5 g	Mean (water control group): 0.4 g Range (water control group): 0.22 to 0.68 g
Mean Standard Length Longest not > 2x shortest	Mean (water control group): 34.0 mm Range (water control group): 29 to 42 mm
Supplier	Osage Catfisheries, Osage Beach, MO
All fish from same source?	yes
All fish from the same year class?	yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 14 days	approx. 11 weeks
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	authors reported that over 2 wk period preceding test fish appeared in good physical condition
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	48 hrs prior to test food withheld; no feeding during test

Guideline Criteria	Reported Information
Pretest Mortality < 3% mortality 48 hours prior to testing	not reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	supplied from wells adjacent to the lab; water is passed through birm filters to remove iron; electrodialysis unit is used to remove approx. 50% of the minerals; water is then passed over an aerator to remove excess CO_2; water is then stored in underground tanks before being pumped into lab
Does water support test animals without observable signs of stress?	authors reported that over 2 week period prior to test fish appeared in good physical condition
Water Temperature 17°C or 22°C	20.9 °C (average during 48 hrs immediately preceding study)
pH Prefer 7.2 to 7.6	ranged 7.0 to 7.5 immediately preceding study
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	near saturation prior to test initiation
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$	122 mg/L as $CaCO_3$ prior to beginning of test
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	18.9 L glass vessels (contained approx. 15 L of test solution)

Guideline Criteria	Reported Information
Type of Dilution System Must provide reproducible supply of toxicant	static test
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.3 g/L in the water control
Photoperiod 16 hours light, 8 hours dark	yes
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	acetone; nominal concentration in acetone control and treatment solutions was 0.5 ml/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	nominal concentrations selected for this study were based upon a pilot range finding study
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	1.0, 2.5, 5.0, 6.5, 8.0, and 9.5 mg/l
Control Mortality ≤ 10% if static, ≤ 5% if flow-through	0% mortality
Number of Test Organisms Minimum 10/level, may be divided among containers	10 fish per concentration level including negative (water) control and solvent (acetone) control

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	yes
Biological observations made every 24 hours?	yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h high, medium, and low doses of control	temperature, pH and DO content in each test vessel were measured and recorded daily; total alkalinity, total hardness, and conductivity were measured at test initiation in the control water
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	yes (at 0 and 96 hrs); from each control and treatment level

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Control Mortality Not more than 10%	0%
Raw data included?	no
Signs of Toxicity (if any) were described?	yes

Guideline Criteria	Reported Information
Physical/Chemical Measurements	water quality measurements were similar in control and treated test solutions; DO ranged 4.3 - 10.6 mg/l; pH ranged 7.0 - 8.5; temperature ranged 21.0 - 22.1 °C; total hardness was 103 mg/l as $CaCO_3$; total alkalinity was 140 mg/l as $CaCO_3$; conductivity was 328 umhos

Comment: The authors reported that at 48 hours the DO concentration had fallen below 60% saturation in the vessels and that thereafter all vessels were gently aerated for the remainder of the study.

B. Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
acetone control	-	10	0	0	0	0
water control	-	10	0	0	0	0
1.0	0.95	10	0	0	0	0
2.5	2.10	10	0	0	0	0
5.0	4.60	10	0	0	0	0
6.5	7.05	10	0	0	1	8
8.0	7.30	10	0	0	5	10
9.5	9.05	10	1	2	7	10

C. Statistical Results

Method: Spearman-Karber method

96-hr LC_{50}: 5.94 mg ai/l 95% C.I.: 5.6 - 6.3 mg ai/L

D. Analytical Results:

The authors reported that concentrations of XDE-105 remained relatively stable over the 96-hour test period, as levels measured at the end of the test ranged from 72% to 88% of those determined at test initiation. The mean measured concentrations averaged 0.95, 2.10, 4.60, 7.05, 7.30, and 9.05 mg/l for nominal concentration levels 1.0, 2.5, 5.0, 6.5, 8.0, and 9.5 mg/l respectively (Table 1, attached).

12. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE:

A GLP statement was included in the report indicating that the study was conducted in accordance with GLP standards. A Quality Assurance Statement was also included.

The authors reported there were no mortalities or signs of toxicity at levels $\leq$ 2.10 mg/l. At the 4.6 and 7.05 mg/l concentration levels there were exposure related signs of toxicity, which included labored respiration and hypoactivity. 100% mortality occurred with fish exposed to the $\geq$ 7.30 mg/l concentration level. Based upon mean measured concentrations, the reported 96-hour LC_{50} was 5.94 mg a.i./l (95% C.I.: 5.60 - 6.30 mg ai/l; slope of mortality curve was 22.8).

13. REVIEWER'S COMMENTS:

Verification of Statistical Results:

Results of the binomial test yielded similar results.

Parameter	Result
Binomial Test LC_{50} (C.I.)	6.16 mg ai/l (4.6- 7.3 mg ai/l)
Moving Average Angle LC_{50} (95% C.I.)	not appropriate
Probit LC_{50} (95% C.I.)	not appropriate
Probit Slope	-
NOEC	2.1 mg ai/l

The following study deviations were noted:

o the mean weight of the fish (0.4 g) was less than the minimum recommended (0.5 g).

o there was no A 15-30 minute transition period between dark and light; this is recommended by the SEP.

o the stock solution was prepared approx. 72 hours prior to test initiation. The SEP recommends that the test be initiated within 30 minutes of solution preparation.

o fish mortality during the acclimation period was not reported.

o the physical characteristics of XDE-105 were not described. In correspondence from DowElanco to A. Heyward dated February 15, 1995 it is indicated that the test material used in this study was a solid material, light grey to white in color.

Adequacy of Study:

1. Classification: Core
2. Rationale: N/A
3. Reparability: N/A

14. Completion Date of One-Liner for Study:

jedwards spinosed bluegill

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
9.05	10	10	100	9.765625E-02
7.3	10	10	100	9.765625E-02
7.05	10	8	80	5.46875
4.6	10	0	0	9.765625E-02
2.1	10	0	0	9.765625E-02
.95	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 4.6 AND 7.3 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 6.163462

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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DER dated 3/24/95 (MRID 43414534)

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