US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD
FRESHWATER INVERTEBRATE LC_{50} TEST
GUIDELINE 72-2

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

   Shaughnessey #: 110003

2. TEST MATERIAL: XDE-105; AGR293707; Lot ACD13651; 88% potency as combined compounds (76.1% and 11.9% factors A and D, respectively); water solubility: approx. 235 mg/l at pH 7 (Factor A)off-white powder

3. CITATION: D. P. Milazzo, H.D. Kirk, M.F. Servinski and J.M. Hugo. 1994. XDE-105(LOT# ACD 13651): Evaluation of the Acute Toxicity to the Daphnid, Daphnia magna Straus. EPA Guideline No. 72-2; Lab. Study No. DECO-ES-2736B; Environmental Toxicology and Chemistry Research Laboratory, Dow Chemical Co., Midland, MI 48674; Submitted by DowElanco, Indianapolis, IN, 46268; MRID 43414537

4. REVIEWED BY:

   Joanne S. Edwards
   Entomologist
   Ecological Effects Branch
   Environmental Fate and
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   Signature: Joanne S. Edwards
   Date: 3/14/95

5. APPROVED BY:

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

   Signature: L.W.T.
   Date: 3/24/95

6. CONCLUSIONS: This study scientifically sound, and satisfies the guideline requirement for 72-2. Based upon mean measured concentrations, the 48-hour EC_{50} of XDE-105 to daphnids is 14 ppm, which classifies this test material as slightly toxic to aquatic invertebrates.

7. ADEQUACY OF THE STUDY: Core.

8. RATIONAL FOR CLASSIFICATION: N/A

9. BACKGROUND: New chemical EUP submission.

10. MATERIALS AND METHODS

A. Test Organisms:

Guideline Criteria	Reported Information
Species (Scientific Name)	Daphnia magna
All organisms should be approximately the same size and weight.	yes
Immature organism should be used. Daphnids 1st (<24hrs). Amphipods, stoneflies, and mayflies in 2nd instar; midges 2nd & 3th instar	<24 hrs at test initiation
Supplier	in-house culture
All organisms from same source (yes or no)	yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 7 days)	source of test organisms was stock tanks with daphnids greater than 14 days old which had at least 4 broods
Wild caught 7 day quarantine (yes or no)	N/A
Check for signs of disease or injury (yes or no, if yes describe)	none indicated
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	N/A
No feeding during the study (When last fed)	not fed during test
<3% mortality 48 hours prior to testing (% mortality, if any)	not reported

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (prefer soft reconstituted water)	water obtained from upper Saginaw Bay of Lake Huron off Whitestone Point; water was adjusted to hardness of about 170 mg/l as $CaCO_3$
Does water support test animals without observable signs of stress?	yes (no control mortality during the test)
Was dechlorinated water used (not recommended)	no
Water Temperature (Daphnia-20°C) (Amphipods and mayflies-17°C) (Midges and mayflies-22°C) (Stoneflies-12°C)	stock cultures maintained at 20 ± 2°C
pH	ranged 6.9 to 7.9 during study
Dissolved Oxygen (Static 1st 48 hrs 60%; 2nd 48 hrs 40%; Flow-through 60%) (% of lowest conc. & hour)	DO in test vessels was >89% of saturation during the study
Total hardness (40 to 48 mg/L as $CaCO_3$ well water)	164 mg/L as $CaCO_3$
Test Aquaria 1. Material (glass or stainless steel) 2. a. Small organisms (3.9 L (1 gal) with 2 to 3 L solution) b. Daphnids and midges (250 ml glass beakers 200mls of test solution)	test vessels were sterilized 250 mL borosilicate glass beakers containing 200 mL of test solution or dilution water
Type of Dilution System (Reproducible supply of toxicant)	static test
Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	N/A

Biomass Loading Rate (Static no > 0.8 g/L ≤ 17°C; >17°C 0.5g/L; Flow-through 1 g/L/24 & must not be >10 g/L at any time at or below 17°C or 5 g/L at higher temperatures.	not reported
Photoperiod (16 L & 8 D)	16 hours light/8 hours dark
Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow-through)	none used

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 fish, no definitive test required.)	not indicated
Definitive Test Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	0.0277, 0.0395, 0.0564, 0.0805, 0.115, 0.164, 0.234, 0.334, 0.477, 0.681, 0.973, 1.39, 1.99, 2.84, 4.05, 5.78, 8.26, 11.8, 16.8, 24, 34.3, 49, 70, and 100 mg/L
Preparation of Stock Solution	primary stock solutions were prepared on day 0 and day 1; were adjusted to pH of approx. 3.5, and stirred overnight to completely dissolve the test material; daphnids were placed in test vessels within 1/2 hr after the test solutions were poured into their designated test vessels; test solutions were renewed and daphnids transferred to the new solutions after 24 hrs of exposure

Controls (Minimum control mortality; static 10%; flow-through 5%	there were two negative (dilution water) control; 0% mortality during test
Number of Test Organisms; (Minimum 20/level can be divided among containers)	20/test concentration including water control; 2 replicates per treatment level
All organisms must be randomly assigned to test vessels (yes or no, describe if no)	impartially added to test vessels
Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	temperature, DO and pH was recorded in high, middle and low exposure concentrations, including the control, at test initiation and every 24-hr interval; temperature was continuously monitored in one representative test vessel using a chart recorder; dilution water was monitored weekly for pH, alkalinity, conductivity and hardness and 3X per year for total suspended solids, total organic carbon, and selected inorganic constituents and organic compounds
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	yes; at day 0, 1 and 2 on each test concentration and control; samples were also taken on day 1 and day 2 during the test period from the "spent" test solutions and controls

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	0.021, 0.0269, 0.0411, 0.0585, 0.0846, 0.1333, 0.196, 0.303, 0.451, 0.633, 0.883, 1,28, 1.84, 2,7, 3.91, 5.69, 8.09, 11.8, 16.6, 23.7, 33.5, 48.2, 68.5, and 96.4 mg/l

Recovery of Chemical (% recovery)	the percent of target values for all dosing solutions ranged from 84.1% to 107% (Table 6, attached); the measured concentrations were based upon the average of four daily measurements (day 0 and day 1 dosing solutions and day 1 and day 2 "spent" test solutions.
Water Quality Measurements	during study temperature ranged 19.7 to 21 ° C; pH ranged 6.9 to 7.9; DO ranged 8 to 10.3 mg/L; conductivity was 500 umhos/cm; hardness was 164 mg/L $CaCO_2$; alkalinity was 47 mg/L $CaCO_2$
Mortality & Observations (Describe observations & attach mortality tables)	immobility defined as no whole body movement relative to the water within a 15 second period; the authors reported that immobility was observed through 0.451 mg/L.

12. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A GLP statement was included in the report indicating that the study was conducted according to GLP Standards with the following exception: The test substance characterization and subsequent purity assays were not conducted by the testing facility and were not audited for compliance with GLP Standards by the Dow Chemical Co. A Quality Assurance Statement was also included in the report.

A computer program, which included probit analysis, moving average angle analysis and binomial probability/non-linear interpolation was used to calculate the LC_{50} The 48-hour LC_{50} for daphnids exposed to XDE-105 was reported to be 92.67 mg/L. The 48-hour LC_{05} for daphnids exposed to XDE-105 was reported to be 31.22. The 48-hr NOEC for mortality was reported to be 33.5 mg/L.

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure: The procedure was generally in accordance with the ASTM Guideline (E 729-88) and the Agency's assessment guidelines.

B. Statistical Analysis: Results were calculated using the binomial, and took into account both mortality and immobility data (analysis, attached). The 48-hour EC_{50} for daphnids exposed to XDE-105 is 14 mg/L (C.I. 1.84 - 33.5 mg/L).

C. Discussion/Results:

The following deviations were noted:

o the biomass loading rate was not reported.

o the raw data on toxic effects other than mortality (immobility and any other toxic effects) at each level was not included in the original report; the addendum provided to the report (attached to this DER) needs to be assigned an MRID.

14. COMPLETION DATE OF ONE-LINER FOR STUDY:

jedwards spinosed daphnid

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
96.4	20	20	100	9.536742E-05
68.5	20	20	100	9.536742E-05
48.2	20	19	95	2.002716E-03
33.5	20	20	100	9.536742E-05
23.7	20	13	65	13.1588
16.6	20	11	55	41.19014
11.8	20	9	45	41.19014
8.09	20	8	40	25.17223
5.69	20	6	30	5.765915
3.91	20	0	0	9.536742E-05
2.7	20	8	40	25.17223
1.84	20	4	20	.5908966
1.28	20	1	5	2.002716E-03
.883	20	4	20	.5908966
.633	20	3	15	.1288414
.451	20	4	20	.5908966
.303	20	0	0	9.536742E-05
.196	20	1	5	2.002716E-03
.133	20	0	0	9.536742E-05
.0846	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.84 AND 33.5 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 13.99571

DER dated 3/24/95 (MRID 434145337)

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Pages 9 through 20 are not included in this copy.

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_____ Identity of product inert ingredients.

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_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

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_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
