DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4(A)

1. **CHEMICAL:** XDE-105 Shaughnessey #: 11003

2. **TEST MATERIAL:** Spinosad Purity: 88%

3. **CITATION:**
Authors: J.T. Weinberg, Kirk, H.D., Rick, D.L., Martin, M.D.
Title: Evaluation of the Toxicity of XDE-105 to Early Life Stages of the Rainbow Trout, Oncorhynchus mykiss Walbaum
Study Completion Date: 1993
Laboratory: Environmental Toxicology and Chemistry Research Laboratory, Dow Chemical Co., Midland, MI
Laboratory Report ID: Not provided
Sponsor: DowElanco, Indianapolis, IN 46268-1189
MRID No.: 43414541

4. **REVIEWED BY:**

Joanne S. Edwards, Entomologist EEB, EFED, U.S.EPA

Signature: Joanne S Edwards Date: 4/27/96

5. **APPROVED BY:**

Leslie Touart, Section Chief EEB, EFED, U.S.EPA

Signature: L. Touart Date: 3/25/96

6. **CONCLUSIONS:**

Based on a statistically significant reduction in growth (length and weight) at the 3.76 treatment level, a statistically significant reduction in survival at the 1.89 ppm treatment level, and a statistically significant reduction in day to mean hatch at the 0.962 ppm treatment level, the overall NOEC is 0.498 ppm. The MATC is 0.692 ppm.

7. **ADEQUACY OF THE STUDY:**

A: Classification: Core

B. Rationale: N/A

C. Reparability: N/A

8. **MAJOR GUIDELINE DEVIATIONS:** None.

9. **MATERIALS AND METHODS:**

A. **Biological System:**

Guideline Criteria	Reported Information
Species: Any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See SEP for complete listing.	rainbow trout *Oncorhynchus mykiss*
Source	Mt. Lassen Trout Farms, RedBluff, CA
Age at beginning of test: Embryos 2 to 24 hours old.	<48 hr
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for post-hatch exposure.	4 replicates; 50 embryos per replicate (200 per treatment level); embryos were indiscriminately culled down to 25 per replicate on day 17 of exposure.
Post Hatch: % of embryos that produce live fry must be ≥ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	98%
Feeding: Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32.	Alevins >2 days were fed a standard laboratory diet supplemented with newly hatched brine shrimp 4X daily (twice daily on weekends and holidays); larvae were not fed for 24 hrs prior to test termination
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Counts were made at least weekly

Guideline Criteria	Reported Information
Controls: Avg. survival at end of test must be ≥ 80%. Survival in any control chamber must not be < 70%.	93.9%
Controls: Negative control and carrier control (when applicable) are required.	water control only

Comments:

B. Physical System:

Guideline Criteria	Reported Information
Test Water: 1) May be natural or reconstituted; 2) Natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants. 3) Hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 is recommended.	Natural water from Saginaw Bay of Lake Huron- water was sand-filtered, pH-adjusted with CO_2, carbon-filtered and UV-irradiated. Water analyzed 3X yearly for contaminants. Hardness of 73 mg/L as $CaCO_3$ in water control recommended.
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature.	Thermostatic temperature controller set to maintain a temperature of 10 ± 2 °C for embryos and 12 ± 2 °C for fry and elevins
Photoperiod: Recommend 16L/8D.	Embryos were shielded from direct light by polyethylene curtains around the exposure system; once hatching was completed normal laboratory lighting (16 h light/8 hr dark with dawn/dusk transitions) was initiated

Guideline Criteria	Reported Information
Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.	Intermittent flow proportional diluter
Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.	Mixing/splitting chambers were used Aeration not used for mixing Flow splitting accuracy not indicated
Test Vessels: All glass or glass with stainless steel frame.	Aquaria were constructed of double-strength glass and clear silicone adhesive measuring approx. 29.5 x 13.7 x 10 cm fitted with glass covers ; volume of approx. 3.7 L per vessel was maintained
Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	Circular glass cups with 1000 um Nitex screen-covered bottoms ; cups were supported in the test vessels by glass beads.
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain DO at above 75% of saturation and maintain the toxicant level.	6 volume turnovers per day
Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.	Prior to use in the diluter system water was aerated to near 100% saturation

Comments: No comments.

C. Chemical System:

Guideline Criteria	Reported Information
Concentrations: Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate. - Toxicant conc. must be measured in one tank at each toxicant level every week. - One concentration must adversely affect a life stage and one concentration must not affect any life stage.	6 concentrations and a water control; 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 mg/L. 4 replicates; 50 embryos per replicate (200 per treatment level); embryos were indiscriminately culled down to 25 per replicate on day 17 of exposure
Other Variables: 1) DO must be measured at each conc. at least once a week; 2) Freshwater parameters in a control and one concentration must be analyzed once a week.	Yes DO ranged 94.4 - 100% saturation during the study pH ranged 7.2 - 7.8 temperature ranged 10.3 - 10.9 °C
Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	No solvent was employed

Comments:

10. REPORTED RESULTS:

Guideline Criteria	Reported Information
Data Endpoints must include: - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if approp.); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs.	Total number of embryos hatched; number normal larvae hatched; time to hatch; mortality of embryos,larvae, and juveniles; Time to swim-up; measurement of growth; abnormalities
Raw data included? (Y/N)	Yes

Comments: When hatching was about 90% complete the number of

larvae per replicate was counted (dead/deformed were subtracted from the total to determine the normal larvae to hatch. Larvae were observed and counted with mortality/sublethal effects at least weekly. The test continued for 30 days post swim-up of controls. At termination all surviving fish were sacrificed and measurements were taken of length and weight.

Effects Data:

Toxicant Conc. (ppm)		Total Embryos Hatched	Day to Mean Hatch	Survival at Test Terminati on	Total Length (mm)	Wet weight (gm)
Nom.	Meas.					
Ctrl	-	98	32	94	31.6	457.7
0.025	0.251	97	32	96	32.0	476.4
0.5	0.498	97	31.75	98	31.9	464.2
12	0.962	100	31	90	32.1	486.7
2	1.89	99	31	91	31.7	470.2
4	3.76	99	31	70	28.4	326.2
8	7.81	979	30.5	0	-	-

Statistical Results:

Statistical Method: Dunnett's or Steel's test

NOEC: 0.498 ppm (lowest, based on all endpoints tested)
LOEC: 0.962 ppm
MATC: 0.692 ppm

The study authors reported no significant statistical differences were demonstrated for the parameters: % of embryos hatched, % normal larvae at hatch, or % survival to thinning. Statistically significant findings were noted in survival at swim-up in the 7.81 ppm treatment group. Significant findings were noted in the 3.76 to 7.81 ppm treatment levels for overall survival, proportion of larvae that showed sublethal effects and growth reduction (length and weight).

According to the study authors, the most significant finding was a dose-related decrease in the day to mean hatch, which was noted in dose levels down to 0.962 ppm.

11. **Reviewer's Statistical Results:**

The authors' statistical results were not contained in the study report, and so a comparison of the statistical results cannot be made. Statistics were run for growth (weight and length separately), day to mean hatch, and survival, with the following results:

Statistical Method: Dunnett's Test

(length)
NOEC 1.89 ppm LEL: 3.76 MATC: 2.67 ppm

(weight)
NOEC 1.89 ppm LEL: 3.76 MATC: 2.67 ppm

Statistical Method: William's Test- % survival/arcsine transformed

(survival)
NOEC: 0.962 ppm LEL: 1.89 ppm MATC: 1.34 ppm

Statistical Method: Dunnett's Test
(day to mean hatch)
NOEC: 0.498 ppm LEL: 0.962 ppm MATC: 0.692 ppm

Comments:

The hardness of the water (73 mg/L as $CaCO_3$ in the water control) was higher than the recommended hardness for this study (40 to 48 mg/L as $CaCO_3$).

Flow splitting accuracy was not reported; should have been within 10%.

We are not sure what the exact age of the test embryos was, since conflicting statements were made in the report (<24 hrs on pg. 7 and <48 hrs on pg. 12). Embryos aged 2 to 24 hours is recommended.

12. **Conclusions:**

Based on a statistically significant reduction in growth (length and weight) at the 3.76 treatment level, a statistically significant reduction in survival at the 1.89 ppm treatment level, and a statistically significant reduction in day to mean hatch at the 0.962 ppm treatment level,the overall NOEC is 0.498 ppm. The MATC is 0.692 ppm.

12. **COMPLETION OF ONE-LINER FOR STUDY:**

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survival
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	29088.929	4848.155	216.852
Within (Error)	21	469.500	22.357	
Total	27	29558.429		

Critical F value = 2.57 (0.05,6,21)
Since F > Critical F REJECT Ho:All groups equal

survival
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	96.000	96.000		
2	.251	95.000	95.000	0.299	
3	.498	98.000	98.000	-0.598	
4	.962	90.000	90.000	1.795	
5	1.89	82.750	82.750	3.963	*
6	3.76	72.750	72.750	6.954	*
7	7.81	0.000	0.000	28.713	*

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

survival
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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	.251	4	0.932	1.0	1.000
3	.498	4	0.932	1.0	-2.000
4	.962	4	0.932	1.0	6.000
5	1.89	4	0.932	1.0	13.250
6	3.76	4	0.932	1.0	23.250
7	7.81	4	0.932	1.0	96.000

survival
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	96.000	96.000	96.333
2	.251	4	95.000	95.000	96.333
3	.498	4	98.000	98.000	96.333
4	.962	4	90.000	90.000	90.000
5	1.89	4	82.750	82.750	82.750
6	3.76	4	72.750	72.750	72.750
7	7.81	4	0.000	0.000	0.000

survival
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	96.333				
.251	96.333	0.100		1.72	k= 1, v=21
.498	96.333	0.100		1.80	k= 2, v=21
.962	90.000	1.795		1.83	k= 3, v=21
1.89	82.750	3.963	*	1.84	k= 4, v=21
3.76	72.750	6.954	*	1.85	k= 5, v=21
7.81	0.000	28.713	*	1.85	k= 6, v=21

s = 4.728
Note: df used for table values are approximate when v > 20.

survival
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STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	control	96.000				
2	.251	95.000	16.50	10.00	4.00	
3	.498	98.000	21.00	10.00	4.00	
4	.962	90.000	13.00	10.00	4.00	
5	1.89	82.750	10.00	10.00	4.00	*
6	3.76	72.750	10.00	10.00	4.00	*
7	7.81	0.000	10.00	10.00	4.00	*

Critical values use k = 6, are 1 tailed, and alpha = 0.05

length.spin
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	756645.629	126107.605	158.626
Within (Error)	21	16695.020	795.001	
Total	27	773340.649		

Critical F value = 2.57 (0.05,6,21)
Since F > Critical F REJECT Ho:All groups equal

length.spin
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	457.725	457.725		
2	.251	476.375	476.375	-0.935	
3	.498	464.225	464.225	-0.326	
4	.962	486.700	486.700	-1.453	
5	1.89	470.150	470.150	-0.623	
6	3.76	326.225	326.225	6.596	*
7	7.81	0.000	0.000	22.958	*

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

length.spin
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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	.251	4	49.046	10.7	-18.650
3	.498	4	49.046	10.7	-6.500
4	.962	4	49.046	10.7	-28.975
5	1.89	4	49.046	10.7	-12.425
6	3.76	4	49.046	10.7	131.500
7	7.81	4	49.046	10.7	457.725

length.spin
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	457.725	457.725	471.256
2	.251	4	476.375	476.375	471.256
3	.498	4	464.225	464.225	471.256
4	.962	4	486.700	486.700	471.256
5	1.89	4	470.150	470.150	470.150
6	3.76	4	326.225	326.225	326.225
7	7.81	4	0.000	0.000	0.000

length.spin
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	471.256				
.251	471.256	0.679		1.72	k= 1, v=21
.498	471.256	0.679		1.80	k= 2, v=21
.962	471.256	0.679		1.83	k= 3, v=21
1.89	470.150	0.623		1.84	k= 4, v=21
3.76	326.225	6.596	*	1.85	k= 5, v=21
7.81	0.000	22.958	*	1.85	k= 6, v=21

s = 28.196
Note: df used for table values are approximate when v > 20.

weight
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	3395.314	565.886	2151.658
Within (Error)	21	5.525	0.263	
Total	27	3400.839		

Critical F value = 2.57 (0.05,6,21)
Since F > Critical F REJECT Ho:All groups equal

weight
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	31.600	31.600		
2	.251	32.050	32.050	-1.241	
3	.498	31.850	31.850	-0.689	
4	.962	32.075	32.075	-1.310	
5	1.89	31.725	31.725	-0.345	
6	3.76	28.350	28.350	8.962	*
7	7.81	0.000	0.000	87.141	*

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

weight
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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	.251	4	0.892	2.8	-0.450
3	.498	4	0.892	2.8	-0.250
4	.962	4	0.892	2.8	-0.475
5	1.89	4	0.892	2.8	-0.125
6	3.76	4	0.892	2.8	3.250
7	7.81	4	0.892	2.8	31.600

weight
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	31.600	31.600	31.894
2	.251	4	32.050	32.050	31.894
3	.498	4	31.850	31.850	31.894
4	.962	4	32.075	32.075	31.894
5	1.89	4	31.725	31.725	31.725
6	3.76	4	28.350	28.350	28.350
7	7.81	4	0.000	0.000	0.000

weight
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	31.894				
.251	31.894	0.810		1.72	k= 1, v=21
.498	31.894	0.810		1.80	k= 2, v=21
.962	31.894	0.810		1.83	k= 3, v=21
1.89	31.725	0.345		1.84	k= 4, v=21
3.76	28.350	8.961	*	1.85	k= 5, v=21
7.81	0.000	87.125	*	1.85	k= 6, v=21

s = 0.513
Note: df used for table values are approximate when v > 20.

survival
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.000	0.000	0.000
2	.251	0.000	0.000	0.000
3	.498	0.250	0.500	0.250
4	.962	0.000	0.000	0.000
5	1.89	0.000	0.000	0.000
6	3.76	0.000	0.000	0.000
7	7.81	0.333	0.577	0.289

survival
File: C:\STATS\TOXSTAT\~~SURV.SP~~ Day to Mean Hatch Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	8.357	1.393	16.783
Within (Error)	21	1.750	0.083	
Total	27	10.107		

Critical F value = 2.57 (0.05,6,21)
Since F > Critical F REJECT Ho:All groups equal

survival
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	32.000	32.000		
2	.251	32.000	32.000	0.000	
3	.498	31.750	31.750	1.227	
4	.962	31.000	31.000	4.909	*
5	1.89	31.000	31.000	4.909	*
6	3.76	31.000	31.000	4.909	*
7	7.81	30.500	30.500	7.363	*

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

survival

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