8-11-95

435886-04
~~MRID No.: 43295401~~

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

1. **CHEMICAL:** Sulfentrazone (129081)

2. **TEST MATERIAL:** Sulfentrazone technical; 94.2%

3. **CITATION:**

Author:	Boeri, R.L., J.P. Magazu, and T.J. Ward
Title:	Early life-stage toxicity of F6285 technical to the rainbow trout, *Oncorhynchus mykiss*
Date:	1994
Laboratory:	T.R. Wilbury Laboratories, Inc.
Lab. Report #:	308-FM
Sponsor:	FMC Corporation, Princeton, NJ
MRID No.:	435886-04

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature: W. Erickson
Date: 8/09/95

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: Harry Craven
Date: 8/11/95

6. **STUDY PARAMETERS/RESULTS SYNOPSIS:**

Test duration:	99 days
Method:	flow-through test
Type of concentrations:	mean measured
Most sensitive endpoints:	no. survivors 60 days post hatch; fish length (60 days post-hatch)
NOEC:	2.95 mg/l
LOEC:	5.93 mg/l

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirement for an early life-stage study with a freshwater fish.

8. **ADEQUACY OF THE STUDY:** Core.

9. **MAJOR GUIDELINE DEVIATIONS:** None.

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10. **MATERIALS AND METHODS:**

Biological System:

Guideline Criteria	Reported Information
Species:	rainbow trout (*Oncorhynchus mykiss*)
Source	eggs and milt obtained from Mount Lassen Trout Farms, Red Bluff, CA
Age at beginning of test: Embryos 2 to 24 hours old.	embryos ≈2 h post-fertilization at test initiation
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for post-hatch exposure.	40 embryos per replicate, 2 reps. per concentration 30/level
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.	yes <1.6X
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.	dry starter chow for the first 7 days following swim up, then newly hatched *Artemia salina* nauplii 2-3X daily except during the final 40 h of the test
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	recorded daily
Controls: Avg. survival at end of test must be ≥ 80%. Survival in any control chamber must not be < 70%.	93-100% survival in each control and solvent control chamber
Controls: Negative control and carrier control (when applicable) are required.	negative and solvent

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Physical System:

Guideline Criteria	Reported Information
Test Water: 1) well or spring provided the source is not polluted; 2) Water should be sterilized with ultraviolet irradiation and tested for contaminants; 3) Hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 is recommended; 4) Reconstituted water can be used see ASTM.	dechlorinated, filtered tapwater UV sterilized and tested for contaminants 40-48 mg/l pH 7.1-7.2
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature. For rainbow trout, 10°C.	8.7-11.9
Photoperiod: Recommend 16L/8D.	16 h light/8 h dark
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 5 concentrations, with dilution factor of 0.5
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%.	not reported aeration not mentioned not reported not reported
Test Vessels: All glass or glass with stainless steel frame.	20-l glass aquaria containing 15 l test solution

Guideline Criteria	Reported Information
Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	glass cylinders enclosed at one end with Nitex screen
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.	5.9 volume changes per 24 h yes
Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.	yes aeration in test vessels was initiated on the 48th day of exposure to maintain DO at acceptable levels

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.	5 concentrations plus solvent and negative controls; 2 reps measured weekly at each test level yes (see results)
Other Variables: 1) DO must be measured at each conc. at least once a week; 2) Monthly pH range < 0.8 pH units.	DO was measured daily in each test chamber pH was within acceptable limits
Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	DMF (0.5 ml/l)

11. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Data Endpoints must include:	
- Number of embryos hatched;	yes
- Time to hatch;	yes
- Mortality of embryos; larvae, and juveniles;	yes
- Measurement of growth;	yes
- Incidence of pathological or histological effects;	no
- Observations of other effects or clinical signs.	yes
Raw data included? (Y/N)	yes

Effects Data:

Tox. conc. (mg/l) Nom.	Meas.	Rep.	No. embryos hatched[1]	Time to hatch (days)	Survival 60-days post-hatch[2]	Length (mm)	Wet wt. (g)
ctrl	-	1	39	35.7	14	39.8	0.88
		2	37	35.1	15	39.1	0.81
solv ctrl	-	1	36	34.4	14	40.3	0.86
		2	39	34.2	15	40.7	0.87
3	2.95	1	37	35.7	14	40.9	1.03
		2	33	34.8	15	39.6	0.97
6	5.93	1	35	35.3	8	38.7	0.81
		2	35	35.3	11	36.0	0.74
13	11.8	1	35	34.6	7	38.9	0.82
		2	35	34.8	6	39.7	0.88
25	25.2	1	31	33.9	2	37.9	0.60
		2	36	34.1	1	35.4	0.62
50	48.2	1	20	33.4	0	-	-
		2	22	33.0	0	-	-

[1]based on 40 embryos/rep.

[2]based on 15 fish/rep. after hatch and thinning

Comments: "At the conclusion of the test, there was 96.7% survival in the control, solvent control, and at the 2.95 mg/L F6285, 63.3% survival at 5.93 mg/L, 43.3% survival at 11.8 mg/L, 10.0% survival t 25.2 mg/L, and 0% survival at 48.2 mg/L (survival was significantly less than the controls at 5.93 mg/L). The time to first hatch was not significantly different from the controls at any concentration. The time to swim up and feeding (day 46) were identical for the controls and all treatments. Sublethal effects (lethargy) were observed in test vessels containing 5.93, 11.8, 25.2, and 48.2 mg/L F6285 Technical. The mean length of surviving fish exposed to F6285 Technical was significantly less than the controls at 5.93 mg/L and the wet weight of surviving fish exposed to F6285 Technical was significantly less than the controls at 25.2 mg/L."

Statistical Results:

Methods: Dunnett's test;
Kruskal Wallis test

Most sensitive endpoints: no. survivors 60 days post hatch;
no. healthy fish 60 days post hatch;
length of surviving fish

NOEC: 2.95 mg/l
LOEC: 5.93 mg/l

12. **REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:**

Method: Williams test

Most sensitive endpoints: no. survivors 60 days post hatch;
fish length (60 days post-hatch)

NOEC: 2.95 mg/l
LOEC: 5.93 mg/l

12. **REVIEWER'S COMMENTS/CONCLUSIONS:** The study is scientifically sound and fulfills the guideline requirement for a fish early life-stage toxicity test.

STATISTICAL ANALYSES - TOXSTAT

NUMBER OF SURVIVORS (60 DAYS POST HATCH):

SUMMARY STATISTICS — TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Solvent control	2	14.000	15.000	14.500
2	2.95 ppm	2	14.000	15.000	14.500
3	5.93 ppm	2	8.000	11.000	9.500
4	11.8 ppm	2	6.000	7.000	6.500
5	25.2 ppm	2	1.000	2.000	1.500

SUMMARY STATISTICS — TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Solvent control	0.500	0.707	0.500
2	2.95 ppm	0.500	0.707	0.500
3	5.93 ppm	4.500	2.121	1.500
4	11.8 ppm	0.500	0.707	0.500
5	25.2 ppm	0.500	0.707	0.500

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

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Solvent control	2	14.500	14.500	14.500
2	2.95 ppm	2	14.500	14.500	14.500
3	5.93 ppm	2	9.500	9.500	9.500
4	11.8 ppm	2	6.500	6.500	6.500
5	25.2 ppm	2	1.500	1.500	1.500

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

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Solvent control	14.500				
2.95 ppm	14.500	0.000		2.02	k= 1, v= 5
5.93 ppm	9.500	4.385	*	2.14	k= 2, v= 5
11.8 ppm	6.500	7.016	*	2.19	k= 3, v= 5
25.2 ppm	1.500	11.402	*	2.21	k= 4, v= 5

s = 1.140

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

NO. EMBRYOS HATCHED:

SUMMARY STATISTICS TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLVENT CONTROL	2	36.000	39.000	37.500
2	2.95 MG/L	2	33.000	37.000	35.000
3	5.93	2	35.000	35.000	35.000
4	11.8	2	35.000	35.000	35.000
5	25.2	2	31.000	36.000	33.500
6	48.2	2	20.000	22.000	21.000

SUMMARY STATISTICS TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLVENT CONTROL	4.500	2.121	1.500
2	2.95 MG/L	8.000	2.828	2.000
3	5.93	0.000	0.000	0.000
4	11.8	0.000	0.000	0.000
5	25.2	12.500	3.536	2.500
6	48.2	2.000	1.414	1.000

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT CONTROL	2	37.500	37.500	37.500
2	2.95 MG/L	2	35.000	35.000	35.000
3	5.93	2	35.000	35.000	35.000
4	11.8	2	35.000	35.000	35.000
5	25.2	2	33.500	33.500	33.500
6	48.2	2	21.000	21.000	21.000

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT CONTROL	37.500				
2.95 MG/L	35.000	1.179		1.94	k= 1, v= 6
5.93	35.000	1.179		2.06	k= 2, v= 6
11.8	35.000	1.179		2.10	k= 3, v= 6
25.2	33.500	1.886		2.12	k= 4, v= 6
48.2	21.000	7.778	*	2.13	k= 5, v= 6

s = 2.121

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

LENGTH DATA:

SUMMARY STATISTICS TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Solvent control	29	35.600	44.400	40.483
2	2.95 ppm	29	35.600	44.300	40.217
3	5.93 ppm	19	33.000	42.500	37.142
4	11.8 ppm	13	32.500	43.600	39.308
5	25.2 ppm	3	31.300	44.500	37.067

SUMMARY STATISTICS TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Solvent control	2.948	1.717	0.319
2	2.95 ppm	6.429	2.536	0.471
3	5.93 ppm	9.647	3.106	0.713
4	11.8 ppm	10.847	3.294	0.913
5	25.2 ppm	45.643	6.756	3.901

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Solvent control	29	40.483	40.483	40.483
2	2.95 ppm	29	40.217	40.217	40.217
3	5.93 ppm	19	37.142	37.142	38.022
4	11.8 ppm	13	39.308	39.308	38.022
5	25.2 ppm	3	37.067	37.067	37.067

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Solvent control	40.483				
2.95 ppm	40.217	0.370		1.67	k= 1, v=88
5.93 ppm	38.022	3.050	*	1.75	k= 2, v=88
11.8 ppm	38.022	2.697	*	1.77	k= 3, v=88
25.2 ppm	37.067	2.060	*	1.78	k= 4, v=88

s = 2.734

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

WET WEIGHT:

SUMMARY STATISTICS — TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Solvent control	29	0.730	1.190	0.864
2	2.95 ppm	29	0.700	1.500	1.003
3	5.93 ppm	19	0.500	1.140	0.769
4	11.8 ppm	13	0.370	1.230	0.849
5	25.2 ppm	3	0.310	0.880	0.603

SUMMARY STATISTICS — TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Solvent control	0.015	0.121	0.023
2	2.95 ppm	0.033	0.182	0.034
3	5.93 ppm	0.042	0.205	0.047
4	11.8 ppm	0.053	0.231	0.064
5	25.2 ppm	0.081	0.285	0.165

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

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Solvent control	29	0.864	0.864	0.933
2	2.95 ppm	29	1.003	1.003	0.933
3	5.93 ppm	19	0.769	0.769	0.802
4	11.8 ppm	13	0.849	0.849	0.802
5	25.2 ppm	3	0.603	0.603	0.603

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

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Solvent control	0.933				
2.95 ppm	0.933	1.454		1.67	k= 1, v=88
5.93 ppm	0.802	1.162		1.75	k= 2, v=88
11.8 ppm	0.802	1.028		1.77	k= 3, v=88
25.2 ppm	0.603	2.370	*	1.78	k= 4, v=88

s = 0.181

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