

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

1. CHEMICAL: Fenthion (053301)
2. TEST MATERIAL: Baytex technical; 96.9%
3. CITATION:

Author: Surprenant, D.C.
Title: The toxicity of technical grade fenthion
(trade name Baytex) to rainbow trout (*Salmo
gairdneri*) embryos and larvae
Date: 1988
Laboratory: Springborn Life Sciences, Inc., Wareham, MA
Lab. Report #: 88-1-2602
Sponsor: Mobay Corporation, Stilwell, KS
MRID No.: 405641-02

4. REVIEWED BY:

William Erickson
Biologist
EEB/EFED/EPA

Signature: *W. Erickson*

Date: *2/07/96*

5. APPROVED BY:

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: *Harry T. Craven*

Date: *3/15/96*

6. STUDY PARAMETERS/RESULTS SYNOPSIS:

Species: *Oncorhynchus mykiss*
Test duration: 88 days
Method: flow-through
Type of concentrations: mean measured
Most sensitive endpoint: time to hatch
NOEC: 7.5 ppb
LOEC: 15 ppb

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.
10. MATERIALS AND METHODS:

Biological System:

Guideline Criteria	Reported Information
Species:	<i>Oncorhynchus mykiss</i>
Source	Mount Lassen Trout Farm, Red Bluff, CA
Age at beginning of test: Embryos 2 to 24 hours old.	embryos \leq 3-h post- fertilization at test initiation
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treat- ment for post-hatch exposure.	50 embryos per cup; 2 cups per concentration 20/level
Post Hatch: % of embryos that produce live fry must be \geq 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.	larvae fed <i>Artemia salina</i> nauplii 2-3X daily
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	2X weekly
Controls: Avg. survival at end of test must be \geq 80%. Survival in any control chamber must not be < 70%.	\geq 85% survival in all control chambers
Controls: Negative control and carrier control (when applicable) are required.	negative and solvent

Physical System:

Guideline Criteria	Reported Information
<p>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 CaCO₃ and pH of 7.2 to 7.6 is recommended; 4) Reconstituted water can be used see ASTM.</p>	<p>well water not reported 26-36 mg/l pH 7.0-7.3</p>
<p>Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature. For rainbow trout, 10°C.</p>	<p>12°C</p>
<p>Photoperiod: Recommend 16L/8D.</p>	<p>16L/8D</p>
<p>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.</p>	<p>modified proportional diluter 5 concentrations, with dilution factor of 0.5</p>
<p>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%.</p>	<p>yes not reported if aeration used for mixing yes</p>
<p>Test Vessels: All glass or glass with stainless steel frame.</p>	<p>glass aquaria (39 x 20 x 25 cm) containing 15 l test solution</p>
<p>Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</p>	<p>glass jars (5-cm outside diam., 8 cm high) with 16-mesh Nitex screen bottoms</p>

Guideline Criteria	Reported Information
<p>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.</p>	<p>90% replacement in approx. 8 h</p> <p>not reported</p>
<p>Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.</p>	<p>yes</p>

Chemical System:

Guideline Criteria	Reported Information
<p>Concentrations: Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</p> <ul style="list-style-type: none"> - 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. 	<p>5 concentrations plus solvent and negative controls; 2 reps</p> <p>measured weekly at each test level</p> <p>yes</p>
<p>Other Variables:</p> <ol style="list-style-type: none"> 1) DO must be measured at each conc. at least once a week; 2) Monthly pH range < 0.8 pH units. 	<p>measured daily</p> <p>pH ranged from 6.6 to 7.7-7.9 at all concentrations</p>
<p>Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p>	<p>TEG</p>

11. REPORTED RESULTS:

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

Effects Data:

Tox. conc. (ppb) Nom. Meas.	Rep.	% embryos hatched	Time to hatch (days)	Survival 62-days post-hatch	Length (mm)	Wet wt. (g)	
ctrl	<0.9	1	98	24.3	100	65	2.4394
		2	98	25.3	95	64	2.3450
solv ctrl	<0.9	1	91	25.5	100	61	2.0962
		2	93	25.5	85	63	2.3942
1.9	1.6	1	96	25.4	100	61	2.0714
		2	83	25.6	100	62	2.2074
3.8	2.7	1	94	25.3	95	63	2.3273
		2	86	25.5	100	61	2.1133
7.5	5.9	1	90	24.9	100	64	2.4734
		2	92	25.2	95	64	2.4322
15	13	1	92	24.5	95	63	2.2833
		2	93	25.2	95	61	2.0893
30	27	1	89	25.0	100	62	2.2157
		2	96	25.0	100	59	2.0060

Comments: The study author notes that the significant effect on body length at 27 ppb ". . . is linked to observations during preliminary testing where 100% of the larvae exposed

for 144 hours to nominal concentrations $\geq 20 \mu\text{g/L}$ exhibited behavioral anomalies, e.g., complete or partial loss of equilibrium. At lower concentrations, $10 \mu\text{g/L}$ and $5 \mu\text{g/L}$ technical grade Fenthion, no adverse effects were observed. Based on these observations, the growth effect is considered a valid but weak expression of Fenthion toxicity."

Statistical Results:

Method: ANOVA and Williams' test
Most sensitive
endpoint: larval growth (length)
NOEC: 13 ppb
LOEC: 27 ppb
MATC: 19 ppb (geometric mean)

12. VERIFICATION OF STATISTICAL RESULTS: (results attached)

Method: Williams' test
Most sensitive
endpoint: time to hatch (days)
NOEC: 7.5 ppb
LOEC: 15 ppb

13. REVIEWER'S COMMENTS/CONCLUSIONS: The study is scientifically sound and fulfills the guideline requirement for a fish early life-stage toxicity test. The most sensitive measurement endpoint is time to hatch.

STATISTICAL RESULTS

PERCENT EMBRYOS HATCHED:

Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLVENT CONTROL	2	1.429	1.429	1.429
2	1.6 ppb	2	1.146	1.369	1.258
3	2.7 ppb	2	1.187	1.323	1.255
4	5.9 ppb	2	1.249	1.284	1.267
5	13 ppb	2	1.284	1.303	1.294
6	27 ppb	2	1.233	1.369	1.301

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLVENT CONTROL	0.000	0.000	0.000
2	1.6 ppb	0.025	0.158	0.112
3	2.7 ppb	0.009	0.096	0.068
4	5.9 ppb	0.001	0.025	0.017
5	13 ppb	0.000	0.013	0.009
6	27 ppb	0.009	0.097	0.068

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT CONTROL	2	0.980	1.429	1.429
2	1.6 ppb	2	0.895	1.258	1.275
3	2.7 ppb	2	0.900	1.255	1.275
4	5.9 ppb	2	0.910	1.267	1.275
5	13 ppb	2	0.925	1.294	1.275
6	27 ppb	2	0.925	1.301	1.275

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT CONTROL	1.429				
1.6 ppb	1.275	1.791		1.94	k= 1, v= 6
2.7 ppb	1.275	1.791		2.06	k= 2, v= 6
5.9 ppb	1.275	1.791		2.10	k= 3, v= 6
13 ppb	1.275	1.791		2.12	k= 4, v= 6

27 ppb 1.275 1.791 2.13 k= 5, v= 6

s = 0.086 Note: df used for table values are approximate when v > 20.

PERCENT LARVAL SURVIVAL:

Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLVENT CONTROL	2	1.345	1.459	1.402
2	1.6 ppb	2	1.459	1.459	1.459
3	2.7 ppb	2	1.345	1.459	1.402
4	5.9 ppb	2	1.345	1.459	1.402
5	13 ppb	2	1.345	1.345	1.345
6	27 ppb	2	1.459	1.459	1.459

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLVENT CONTROL	0.006	0.080	0.057
2	1.6 ppb	0.000	0.000	0.000
3	2.7 ppb	0.006	0.080	0.057
4	5.9 ppb	0.006	0.080	0.057
5	13 ppb	0.000	0.000	0.000
6	27 ppb	0.000	0.000	0.000

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT CONTROL	2	0.975	1.402	1.402
2	1.6 ppb	2	1.000	1.459	1.402
3	2.7 ppb	2	0.975	1.402	1.402
4	5.9 ppb	2	0.975	1.402	1.402
5	13 ppb	2	0.950	1.345	1.402
6	27 ppb	2	1.000	1.459	1.459

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT CONTROL	1.402				
1.6 ppb	1.402	0.000		1.94	k= 1, v= 6
2.7 ppb	1.402	0.000		2.06	k= 2, v= 6

5.9 ppb	1.402	0.000	2.10	k= 3, v= 6
13 ppb	1.402	0.000	2.12	k= 4, v= 6
27 ppb	1.459	1.000	2.13	k= 5, v= 6

s = 0.057 Note: df used for table values are approximate when v > 20.

LARVAL LENGTH 62-DAYS POSTHATCH:

Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLVENT CONTROL	2	64.000	65.000	64.500
2	1.6 ppb	2	61.000	62.000	61.500
3	2.7 ppb	2	61.000	63.000	62.000
4	5.9 ppb	2	64.000	64.000	64.000
5	13 ppb	2	61.000	63.000	62.000
6	27 ppb	2	59.000	62.000	60.500

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLVENT CONTROL	0.500	0.707	0.500
2	1.6 ppb	0.500	0.707	0.500
3	2.7 ppb	2.000	1.414	1.000
4	5.9 ppb	0.000	0.000	0.000
5	13 ppb	2.000	1.414	1.000
6	27 ppb	4.500	2.121	1.500

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT CONTROL	2	64.500	64.500	64.500
2	1.6 ppb	2	61.500	61.500	62.500
3	2.7 ppb	2	62.000	62.000	62.500
4	5.9 ppb	2	64.000	64.000	62.500
5	13 ppb	2	62.000	62.000	62.000
6	27 ppb	2	60.500	60.500	60.500

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT CONTROL	64.500				

1.6 ppb	62.500	1.589	1.94	k= 1, v= 6
2.7 ppb	62.500	1.589	2.06	k= 2, v= 6
5.9 ppb	62.500	1.589	2.10	k= 3, v= 6
13 ppb	62.000	1.987	2.12	k= 4, v= 6
27 ppb	60.500	3.179	2.13	k= 5, v= 6

s = 1.258 Note: df used for table values are approximate when v > 20.

LARVAL WEIGHT 62-DAYS POSTHATCH:

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLVENT CONTROL	2	2.096	2.394	2.245
2	1.6 ppb	2	2.071	2.207	2.139
3	2.7 ppb	2	2.113	2.327	2.220
4	5.9 ppb	2	2.432	2.473	2.453
5	13 ppb	2	2.089	2.283	2.186
6	27 ppb	2	2.006	2.216	2.111

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLVENT CONTROL	0.044	0.211	0.149
2	1.6 ppb	0.009	0.096	0.068
3	2.7 ppb	0.023	0.151	0.107
4	5.9 ppb	0.001	0.029	0.021
5	13 ppb	0.019	0.137	0.097
6	27 ppb	0.022	0.148	0.105

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT CONTROL	2	2.245	2.245	2.264
2	1.6 ppb	2	2.139	2.139	2.264
3	2.7 ppb	2	2.220	2.220	2.264
4	5.9 ppb	2	2.453	2.453	2.264
5	13 ppb	2	2.186	2.186	2.186
6	27 ppb	2	2.111	2.111	2.111

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
----------------	-----------------	----------------	-----------	----------------	--------------------

SOLVENT CONTROL	2.264				
1.6 ppb	2.264	0.137	1.94	k= 1, v= 6	
2.7 ppb	2.264	0.137	2.06	k= 2, v= 6	
5.9 ppb	2.264	0.137	2.10	k= 3, v= 6	
13 ppb	2.186	0.420	2.12	k= 4, v= 6	
27 ppb	2.111	0.957	2.13	k= 5, v= 6	

s = 0.140 Note: df used for table values are approximate when v > 20.

TIME TO HATCH:

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Solvent control	2	25.500	25.500	25.500
2	1.9	2	25.400	25.600	25.500
3	3.8	2	25.300	25.500	25.400
4	7.5	2	24.900	25.200	25.050
5	15	2	24.500	25.200	24.850
6	30	2	25.000	25.000	25.000

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Solvent control	0.000	0.000	0.000
2	1.9	0.020	0.141	0.100
3	3.8	0.020	0.141	0.100
4	7.5	0.045	0.212	0.150
5	15	0.245	0.495	0.350
6	30	0.000	0.000	0.000

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Solvent control	2	25.500	25.500	25.500
2	1.9	2	25.500	25.500	25.500
3	3.8	2	25.400	25.400	25.400
4	7.5	2	25.050	25.050	25.050
5	15	2	24.850	24.850	24.925
6	30	2	25.000	25.000	24.925

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISOTONIZED CALC. SIG TABLE DEGREES OF

IDENTIFICATION	MEAN	WILLIAMS	P=.05	WILLIAMS	FREEDOM
Solvent control	25.500				
1.9	25.500	0.000		1.94	k= 1, v= 6
3.8	25.400	0.426		2.06	k= 2, v= 6
7.5	25.050	1.919		2.10	k= 3, v= 6
15	24.925	2.452	*	2.12	k= 4, v= 6
30	24.925	2.452	*	2.13	k= 5, v= 6

s = 0.235 Note: df used for table values are approximate when v > 20.