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

1. **CHEMICAL:** Fonophos (041701)

2. **TEST MATERIAL:** Fonophos technical; 95.9%

3. **CITATION:**

Author:	Kent, S.J., S.A. Sankey, J.E. Caunter, and P.A. Johnson
Title:	Fonofos: chronic toxicity to sheepshead minnow (*Cyprinodon variegatus*) embryos and larvae.
Date:	1995
Laboratory:	Brixham Environmental Laboratory, Brixham Devon, UK
Lab. Report #:	BL5374/B
Sponsor:	ZENECA Agrochemicals, Surrey, UK
MRID No.:	436323-01

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature: W. Erickson
Date: 10/27/95

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: Harry Craven
Date: 11/3/95

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

Test duration:	37 days
Method:	flow through
Type of concentrations:	mean measured
Most sensitive endpoint:	post-hatch survival
NOEC:	2.7 µg ai/l
LOEC:	5.3 µg ai/l

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirement for an early life-stage study with an estuarine/marine 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:	sheepshead minnow (*Cyprinodon variegatus*)
Source	in-house
Age at beginning of test: Embryos 2 to 24 hours old.	<24 h old
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for post-hatch exposure.	20 embryos per replicate, 4 reps. per concentration 72-79 per 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.	<24 h old *Artemia salina* 2-3X daily except during the final 24 h of the test; from day 11 post-hatch, high protein pelleted food replaced one of the daily *Artemia* feedings
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%.	86-90% survival in control and solvent groups; 80-95% in individual reps
Controls: Negative control and carrier control (when applicable) are required.	negative and solvent (DMF)

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.	a mix of 75% seawater and 25% dechlorinated tap water yes 41.6-49 mg/l pH 7.9-8.2
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature.	24.2-25.4°C
Photoperiod: Recommend 16L/8D.	16L/8D
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.	not reported, but diagram appears to be a serial 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%.	yes aeration not mentioned not reported yes
Test Vessels: All glass or glass with stainless steel frame.	11.8-l glass vessels containing 9.5 l test solution
Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	5 x 8-cm glass tubing with nylon mesh (0.47 mm^2) cemented to the bottom by silicone sealant

Guideline Criteria	Reported Information
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 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 not aerated during testing

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; 4 reps measured weekly at each test level yes
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 twice weekly 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 (0.1 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. (µg ai/l) Nom.	Meas.	Rep.	% embryos hatched[1]	Time to hatch (days)	% Survival 32-days post-hatch	Length (mm)	Wet wt. (g)
ctrl	<0.1	1	100	4.9	90	17.8	165.0
		2	95	4.9	84	17.6	162.9
		3	100	5.2	90	17.4	153.3
		4	100	5.0	95	17.2	123.3
solv ctrl	<0.1	1	100	6.0	80	18.2	183.8
		2	100	5.9	95	18.0	169.2
		3	100	5.9	80	17.2	154.3
		4	95	5.9	89	17.6	178.4
1.25	1.7	1	95	5.9	100	18.4	184.8
		2	85	5.9	94	18.6	206.0
		3	85	5.8	100	18.8	191.8
		4	95	6.0	89	19.0	211.2
2.5	2.7	1	95	5.9	95	18.3	188.3
		2	95	5.8	100	18.0	184.3
		3	95	5.3	95	18.5	182.9
		4	100	5.7	100	18.0	181.3

5.0	5.3	1	100	5.9	75	18.6	226.0
		2	95	5.9	84	17.4	190.8
		3	85	5.9	76	17.8	217.4
		4	80	5.9	75	18.0	227.1
10	12	1	95	5.9	26	13.6	91.5
		2	100	6.0	15	13.7	96.8
		3	95	5.9	11	17.3	208.1
		4	90	6.0	11	14.2	118.4
20	21	1	95	5.7	0	-	-
		2	95	5.6	5	6.3	8.4
		3	100	5.9	0	-	-
		4	90	5.7	0	-	-

[1]based on 20 embryos/rep.

Toxicity observations: Larvae in the 5.3 µg ai/l treatment group were sluggish and displayed a loss of balance during the test. No other symptoms of toxicity were reported.

Statistical Results:

Methods: Contingency tables (% hatch, % survival); Dunnett's test (length, weight)

Most sensitive endpoints: % hatch; post-hatch survival

NOEC: 2.7 µg ai/l LOEC: 5.3 µg ai/l

12. REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Williams' test (results attached)

Most sensitive endpoint: post-hatch survival

NOEC: 2.7 µg ai/l LOEC: 5.3 µg ai/l

Other endpoints:	embryos hatched	time to hatch	length	weight
NOEC (µg ai/l):	21	12	5.3	5.3
LOEC (µg ai/l):	>21	21	12	12

13. **REVIEWER'S COMMENTS/CONCLUSIONS:** Approximately 80% of the dilution water control larvae hatched on day 5, versus only 5-34% for the other groups. This difference was not considered to be caused by the active ingredient, because the response of solvent control group was similar to that of the exposure groups. Data from the dilution water control group were excluded from the reviewer's statistical analyses; thus, percent hatch is not considered as sensitive an endpoint as post-hatch survival. The study is scientifically sound and fulfills the guideline requirement (74-4a) for a fish early life-stage toxicity test.

REVIEWER'S STATISTICAL RESULTS

% EMBRYOS HATCHED: Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	CONTROL	4	1.345	1.459	1.430
2	1.7 PPB	4	1.173	1.345	1.302
3	2.7	4	1.345	1.459	1.374
4	5.3	4	1.107	1.459	1.271
5	12	4	1.249	1.459	1.350
6	21	4	1.249	1.459	1.350

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	CONTROL	0.003	0.057	0.028
2	1.7 PPB	0.007	0.086	0.043
3	2.7	0.003	0.057	0.028
4	5.3	0.026	0.160	0.080
5	12	0.007	0.086	0.043
6	21	0.007	0.086	0.043

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	4	0.988	1.430	1.430
2	1.7 PPB	4	0.925	1.302	1.338
3	2.7	4	0.962	1.374	1.338
4	5.3	4	0.900	1.271	1.323
5	12	4	0.950	1.350	1.323
6	21	4	0.950	1.350	1.323

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	1.430			1.73	k= 1, v=18
1.7 PPB	1.338	1.374		1.82	k= 2, v=18
2.7	1.338	1.374		1.85	k= 3, v=18
5.3	1.323	1.590		1.86	k= 4, v=18
12	1.323	1.590		1.87	k= 5, v=18
21	1.323	1.590			

s = 0.095 Note: df used for table values are approximate when v > 20.

TIME TO HATCH: Transform: NO TRANSFORMATION

SUMMARY STATISTICS TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLV CONTROL	4	5.900	6.000	5.925
2	1.7	4	5.800	6.000	5.900
3	2.7	4	5.300	5.900	5.675
4	5.3	4	5.900	5.900	5.900
5	12	4	5.900	6.000	5.950
6	21	4	5.600	5.900	5.725

SUMMARY STATISTICS TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLV CONTROL	0.002	0.050	0.025
2	1.7	0.007	0.082	0.041
3	2.7	0.069	0.263	0.131
4	5.3	0.000	0.000	0.000
5	12	0.003	0.058	0.029
6	21	0.016	0.126	0.063

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLV CONTROL	4	5.925	5.925	5.925
2	1.7	4	5.900	5.900	5.900
3	2.7	4	5.675	5.675	5.842
4	5.3	4	5.900	5.900	5.842
5	12	4	5.950	5.950	5.842
6	21	4	5.725	5.725	5.725

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLV CONTROL	5.925				
1.7	5.900	0.277		1.73	k= 1, v=18
2.7	5.842	0.925		1.82	k= 2, v=18
5.3	5.842	0.925		1.85	k= 3, v=18
12	5.842	0.925		1.86	k= 4, v=18
21	5.725	2.219	*	1.87	k= 5, v=18

s = 0.127

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

% SURVIVAL: Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLV CONTROL	4	1.107	1.345	1.198
2	1.7	4	1.233	1.459	1.368
3	2.7	4	1.345	1.459	1.402
4	5.3	4	1.047	1.159	1.078
5	12	4	0.338	0.535	0.402
6	21	4	0.112	0.226	0.140

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLV CONTROL	0.013	0.115	0.057
2	1.7	0.012	0.111	0.055
3	2.7	0.004	0.066	0.033
4	5.3	0.003	0.054	0.027
5	12	0.009	0.093	0.046
6	21	0.003	0.057	0.028

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLV CONTROL	4	0.860	1.198	1.323
2	1.7	4	0.958	1.368	1.323
3	2.7	4	0.975	1.402	1.323
4	5.3	4	0.775	1.078	1.078
5	12	4	0.158	0.402	0.402
6	21	4	0.013	0.140	0.140

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLV CONTROL	1.323				
1.7	1.323	2.048	*	1.73	k= 1, v=18
2.7	1.323	2.048	*	1.82	k= 2, v=18
5.3	1.078	1.969	*	1.85	k= 3, v=18
12	0.402	13.066	*	1.86	k= 4, v=18
21	0.140	17.365	*	1.87	k= 5, v=18

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

Reviewer's note: the significant difference between the control and treatment means at the 1.7 ppb and 2.7 ppb treatment levels were due to an increase, not a decrease, in survival in the treated groups.

LENGTH: Transform: NO TRANSFORMATION

SUMMARY STATISTICS TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLV CONTROL	4	17.200	18.200	17.750
2	1.7	4	18.400	19.000	18.700
3	2.7	4	18.000	18.500	18.200
4	5.3	4	17.400	18.600	17.950
5	12	4	13.600	17.300	14.700

SUMMARY STATISTICS TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLV CONTROL	0.197	0.443	0.222
2	1.7	0.067	0.258	0.129
3	2.7	0.060	0.245	0.122
4	5.3	0.250	0.500	0.250
5	12	3.073	1.753	0.877

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLV CONTROL	4	17.750	17.750	18.225
2	1.7	4	18.700	18.700	18.225
3	2.7	4	18.200	18.200	18.200
4	5.3	4	17.950	17.950	17.950
5	12	4	14.700	14.700	14.700

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLV CONTROL	18.225				
1.7	18.225	0.787		1.75	k= 1, v=15
2.7	18.200	0.745		1.84	k= 2, v=15
5.3	17.950	0.331		1.87	k= 3, v=15
12	14.700	5.051	*	1.88	k= 4, v=15

s = 0.854

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

WEIGHT: Transform: NO TRANSFORMATION

SUMMARY STATISTICS TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLV CONTROL	4	154.300	183.800	171.425
2	1.7	4	184.800	211.200	198.450
3	2.7	4	181.300	188.300	184.200
4	5.3	4	190.800	227.100	215.325
5	12	4	91.500	208.100	128.700

SUMMARY STATISTICS TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLV CONTROL	166.669	12.910	6.455
2	1.7	150.037	12.249	6.124
3	2.7	8.973	2.996	1.498
4	5.3	286.129	16.915	8.458
5	12	2937.300	54.197	27.098

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLV CONTROL	4	171.425	171.425	192.350
2	1.7	4	198.450	198.450	192.350
3	2.7	4	184.200	184.200	192.350
4	5.3	4	215.325	215.325	192.350
5	12	4	128.700	128.700	128.700

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLV CONTROL	192.350				
1.7	192.350	1.111		1.75	k= 1, v=15
2.7	192.350	1.111		1.84	k= 2, v=15
5.3	192.350	1.111		1.87	k= 3, v=15
12	128.700	2.268	*	1.88	k= 4, v=15

s = 26.642

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