

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD  
ACUTE INVERTEBRATE LIFE-CYCLE TEST  
GUIDELINE 72-4B

1. CHEMICAL: Fonophos (041701)

2. TEST MATERIAL: Fonophos technical; 96%

3. CITATION:

Author: Ward, T.J., J.P. Magazu, and R.L. Boeri  
Title: Chronic toxicity of fonofos to the mysid,  
*Mysidopsis bahia*  
Date: 1995  
Laboratory: T.R. Wilbury Laboratories, Inc.,  
Marblehead, MA  
Lab. Report #: 610-ZE  
Sponsor: Zeneca Limited, United Kingdom  
MRID No.: 436876-01

4. REVIEWED BY:

William Erickson  
Biologist  
EEB/EFED

Signature:



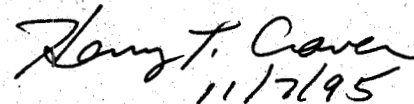
Date:

11/06/95

5. APPROVED BY:

Harry Craven  
Section Head 4  
EEB/EFED

Signature:



Date:

11/7/95

6. STUDY PARAMETERS/RESULTS SYNOPSIS:

Age of Test Organism: <24 h old  
Test Duration: 28 days  
Study Method: Flow through  
Concentrations: Mean measured  
Most sensitive endpoint: Survival  
NOEC: 0.052 ppb  
LOEC: 0.109 ppb

7. CONCLUSIONS: The study is scientifically sound and satisfies the guideline requirement for a chronic toxicity test with an estuarine/marine shrimp.

8. ADEQUACY OF THE STUDY: Core.

9. MAJOR GUIDELINE DEVIATIONS: None.



2043370

10. **MATERIALS AND METHODS:**

**Biological System:**

Guideline Criteria	Reported Information
<b>Species:</b> An estuarine shrimp species, preferably <i>Americamysis bahia</i>	<i>Americamysis bahia</i> (formerly <i>Mysidopsis bahia</i> )
<b>Duration</b> 28 days/one generation	28 days
<b>Source</b> (or supplier)	in-house culture
<b>Parental Acclimation</b> 1) Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids should be in good health.	yes  yes
<b>Parental Acclimation Period</b> At least 14 days	14 days
<b>Chamber Location:</b> Treatments should be randomly assigned to test chamber locations.	yes
<b>Duration of the Test:</b> A mysid test must not be terminated before 7 days past the median time of 1 <sup>st</sup> brood release in the control treatment.	yes
<b>Brood Stock:</b> Test started with mysids: 1) from only one brood stock or 2) from brood stock which has not obtain sexual maturity or had been maintained for > 14 days in a laboratory with same food, water, temperature, and salinity used in the test.	yes

Guideline Criteria	Reported Information
<p><b>Distribution:</b>  <b>No. of mysids before pairing:</b>  Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/level.  <b>No. of mysids after pairing:</b>  ≥ 20 randomly selected pairs/treatment (excess males should be held in separate compartment to replace paired males).</p>	<p>yes</p> <p>20 pairs/treatment</p>
<p><b>Pairing:</b>  1) Should be conducted when most of the mysids are sexually mature (usu. 10-14 days after test initiation).  2) Should be paired on the same day</p>	<p>10 days after exposure</p> <p>yes</p>
<p><b>Feeding:</b>  1) Mysids should be fed live brine shrimp nauplii at least once daily.  2) 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.</p>	<p>fed nauplii 2-3X daily</p> <p>not reported</p>
<p><b>Counts:</b>  Live adult mysids should be counted  1) at initiation,  2) at pairing,  3) and daily after pairing.  4) Live young must be counted and removed daily.  5) Missing or impinged animals should be recorded.</p>	<p>yes</p> <p>yes</p> <p>yes</p> <p>yes</p> <p>yes</p>
<p><b>Controls:</b>  Survival in any control chamber (between pairing and test termination) must not be less than 70%.</p>	<p>&gt;70%</p>

Guideline Criteria	Reported Information
<b>Controls:</b> Negative control and carrier control (when applicable) are required.	no solvent used

**Physical System:**

Guideline Criteria	Reported Information
<b>Test Water:</b> 1) May be natural (sterilized and filtered) or a commercial mixture; 2) During the test, difference between highest and lowest measured salinities must be less than 10 ‰. Should be measured daily. 3) Salinity should be between 15 and 30 ‰. 4) Measured pH should be between 7.6 and 8.2. Must not deviate by more than one unit for more than 48 hours. Should be measured at the beginning, end of test and weekly. 5) Water must be free of pollutants. 6) DO must be measured @ each conc. @ least once a wk. (see details in ASTM)	aerated, filtered, UV-sterilized sea water  <10 ‰; measured daily  15-17 ‰  7.7-8.0  yes  measured daily

Guideline Criteria	Reported Information
<p><b>Test Temperature:</b></p> <p>1) Mean measured temperature for each chamber at test termination should be within 1°C of selected test temperature.</p> <p>2) Each individual measured temperature must be within 3°C of the mean of the time-weighted averages.</p> <p>3) For mysid shrimp, 27°C is recommended.</p> <p>4) Whenever temp. is measured concurrently in more than one test chamber the highest &amp; lowest temp. must not differ by more than 2°C.</p>	<p>within 1.7°C</p> <p>yes</p> <p>25°C</p>
<p><b>Photoperiod:</b> Recommend 16L/8D.</p>	<p>16L/8D</p>
<p><b>Dosing Apparatus:</b></p> <p>1) Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p> <p>2) A minimum of 5 toxicant concentrations</p> <p>3) with a dilution factor not greater than 0.5 and controls should be used.</p>	<p>intermittent flow proportional diluter</p> <p>5 concentrations</p> <p>0.5 dilution factor</p>
<p><b>Toxicant Mixing:</b></p> <p>1) Mixing chamber is recommended but not required;</p> <p>2) Aeration should not be used for mixing;</p> <p>3) It must be demonstrated that the test solution is completely mixed before intro. into the test system;</p> <p>4) Flow splitting accuracy must be within 10%.</p>	<p>not aerated</p> <p>yes</p> <p>yes</p>

Guideline Criteria	Reported Information
<p><b>Test Vessels:</b></p> <p>1) Material: all glass, No. 316 stainless steel, or perflorocarbon plastic</p> <p>2) Size: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume acceptable</p> <p>3) 90 or 140 mm inside dia. glass Petri dish bottoms with collars made of 200 - 250 um mesh screen.</p>	<p>glass aquaria</p> <p>20-l aquaria containg 8 l test solution (4-10 cm deep)</p> <p>100-mm diameter glass perti dishes with Nitex-screen collars attached by silicone adhesive</p>
<p><b>Covers</b></p> <p>1) Renewal: Test vessels should be covered with a glass plate.</p> <p>2) Flow-through: Openings in the test compartments should be covered with nylon mesh or stainless steel screen.</p>	<p>n/a</p> <p>aquaria reported to be loosely covered</p>
<p><b>Flow Rate:</b></p> <p>1) Flow rates should provide 5 to 10 volume additions per 24 hr.</p> <p>2) Flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level.</p> <p>3) Meter systems calibrated before study and checked twice daily during test period</p> <p>4) Renewal must not drop below 50% for more than 48 hours.</p>	<p>8.4 vol. additions per 24 h</p> <p>yes</p> <p>diluter calibrated before and after the test</p> <p>n/a</p>
<p><b>Aeration:</b></p> <p>1) Dilution water should be aerated to insure DO concentration at or near 100% saturation.</p> <p>2) Test tanks may be aerated.</p>	<p>yes</p> <p>aeration was not needed</p>

**Chemical System:**

Guideline Criteria	Reported Information
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<p><b>Concentrations:</b></p> <p>1) Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</p> <p>2) Toxicant conc. must be measured in one tank at each toxicant level every week.</p> <p>3) One concentration must adversely affect a life stage and one concentration must not affect any life stage.</p> <p>4) The measured conc. of the test material of any treatment should be at least 50% of the time-weighted average measured conc. for &gt;10% of the duration of the test.</p> <p>5) The measured conc. for any treatment level should not be more than 30% higher than the time-weighted average measured conc. for more than 5% of the duration of the test.</p>	<p>yes</p> <p>yes</p> <p>yes</p> <p>yes</p> <p>yes</p>
<p><b>Solvents:</b></p> <p>1) Should not exceed 0.1 ml/L in a flow-through system.</p> <p>2) Following solvents are acceptable: triethylene glycol, methanol, acetone, ethanol.</p>	<p>no solvent</p>

**11. REPORTED RESULTS**

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements were included in the report?</p>	<p>yes</p>
<p>1) At least 75% of the paired 1<sup>st</sup> generation females in the control produced young or 2) the average number of young produced by the 1<sup>st</sup> generation females in the control(s) was more than 3.</p>	<p>15.7 young/♀</p>



Guideline Criteria	Reported Information
<b>Data Endpoints</b> must include: 1) Survival of first-generation mysids Female Male 2) Number of live young produced per female 3) Dry weight of each first-generation mysid alive at the end of the test Female Male 4) Length of each 1 <sup>st</sup> generation mysid alive at the end of the study Female Male 5) Incidence of pathological or histological effects; 6) Observations of other effects or clinical signs.	yes (sexes combined)  yes  yes (by sex)  yes (by sex)  no  yes
<b>Raw data included?</b> (Y/N)	yes

**Effects Data:**

Toxicant Conc. ( $\mu\text{g/L}$ )		Mean # Young/♀	% Survival (28 days)	Mean Total Length (mm)			Mean Dry weight (mg)		
Nom.	Meas.			♂	♀	♂ & ♀	♂	♀	♂ & ♀
Ctrl	-	15.7	89	8.1	8.3	8.2	0.81	1.08	0.96
0.031	0.0259	15.8	89	8.0	8.0	8.0	0.87	1.04	0.95
0.065	0.0522	16.0	87	7.9	8.1	8.0	0.84	1.09	0.94
0.13	0.109	16.6	13	-	8.2	-	-	0.86	-
0.25	0.220	11.1	12	-	8.1	-	-	0.77	-
0.50	0.451	0	0	-	-	-	-	-	-

**Sublethal Observations:** Mysids were observed for loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, and change in behavior. Mysids were affected only at the highest test concentration, and all eventually died. Affected mysids swam erratically on days 8-13 and were also noted to be lethargic on days 10-13.

**Statistical Results:**

Endpoint	NOEC ( $\mu\text{g ai/l}$ )	LOEC ( $\mu\text{g ai/l}$ )
Survival-	0.052	0.109
No. young per surviving female	0.220	0.451
Dry weight	0.220	0.451
Length	0.220	0.451

Methods: ANOVA (if variance homogeneous) or  
Kruskall-Wallis' Test (variances heteroscedastic)

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

Endpoint	NOEC ( $\mu\text{g ai/l}$ )	LOEC ( $\mu\text{g ai/l}$ )
Survival	0.052	0.109
No. young per surviving female	0.220	>0.220
Dry weight	0.109	0.220
Length	0.220	>0.220

Method: Williams' Test (results attached)

- 13. REVIEWER'S COMMENTS:** The authors found that survival, with an NOEC of 0.052 ppb, was the most sensitive endpoint. EEB's verification of the data analyses agree with that finding.

Differential mortality of the sexes is indicated by the data. No male mysids survived at test concentrations of 0.109 ppb or higher. Although no females survived dosing at 0.451 ppb, 8 females survived dosing at 0.109 ppb and 7 survived at 0.220 ppb.

The study is scientifically sound and fulfills the guideline requirement for an acute toxicity test with a marine/estuarine shrimp.

STATISTICAL RESULTS - MYSID CHRONIC DATA

SURVIVAL:

Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	CONTROL	2	1.202	1.249	1.225
2	0.0259	2	1.202	1.249	1.225
3	0.0522	2	1.146	1.249	1.197
4	0.109	2	0.174	0.500	0.337
5	0.220	2	0.322	0.369	0.345
6	0.451	2	0.091	0.091	0.091

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	CONTROL	0.001	0.033	0.024
2	0.0259	0.001	0.033	0.024
3	0.0522	0.005	0.073	0.052
4	0.109	0.053	0.231	0.163
5	0.220	0.001	0.033	0.024
6	0.451	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	CONTROL	2	0.885	1.225	1.225
2	0.0259	2	0.885	1.225	1.225
3	0.0522	2	0.865	1.197	1.197
4	0.109	2	0.130	0.337	0.341
5	0.220	2	0.115	0.345	0.341
6	0.451	2	0.000	0.091	0.091

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	1.225				
0.0259	1.225	0.000		1.94	k= 1, v= 6
0.0522	1.197	0.277		2.06	k= 2, v= 6
0.109	0.341	8.713	*	2.10	k= 3, v= 6
0.220	0.341	8.713	*	2.12	k= 4, v= 6
0.451	0.091	11.174	*	2.13	k= 5, v= 6

s = 0.101

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

NO. YOUNG PER SURVIVING FEMALE:

Transform: NO TRANSFORM

SUMMARY STATISTICS

TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	CONTROL	2	14.900	16.400	15.650
2	0.0259	2	15.200	16.400	15.800
3	0.0522	2	11.900	20.000	15.950
4	0.109	2	14.000	19.100	16.550
5	0.220	2	8.700	13.500	11.100

SUMMARY STATISTICS

TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	CONTROL	1.125	1.061	0.750
2	0.0259	0.720	0.849	0.600
3	0.0522	32.805	5.728	4.050
4	0.109	13.005	3.606	2.550
5	0.220	11.520	3.394	2.400

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	2	15.650	15.650	15.988
2	0.0259	2	15.800	15.800	15.988
3	0.0522	2	15.950	15.950	15.988
4	0.109	2	16.550	16.550	15.988
5	0.220	2	11.100	11.100	11.100

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	15.988				
0.0259	15.988	0.098		2.02	k= 1, v= 5
0.0522	15.988	0.098		2.14	k= 2, v= 5
0.109	15.988	0.098		2.19	k= 3, v= 5
0.220	11.100	1.323		2.21	k= 4, v= 5

s = 3.440

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

LENGTH:

Transform: NO TRANSFORMATION

SUMMARY STATISTICS

TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	53	7.100	9.400	8.202
2	0.0259	53	7.600	8.500	8.002
3	0.0522	52	7.500	9.000	8.000
4	0.109	8	7.800	8.700	8.213
5	0.220	7	7.700	8.300	8.086

SUMMARY STATISTICS

TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.258	0.508	0.070
2	0.0259	0.060	0.245	0.034
3	0.0522	0.077	0.277	0.038
4	0.109	0.090	0.300	0.106
5	0.220	0.045	0.212	0.080

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	53	8.202	8.202	8.202
2	0.0259	53	8.002	8.002	8.020
3	0.0522	52	8.000	8.000	8.020
4	0.109	8	8.213	8.213	8.020
5	0.220	7	8.086	8.086	8.020

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	8.202				
0.0259	8.020	2.626	*	1.66	k= 1, v=168
0.0522	8.020	2.614	*	1.73	k= 2, v=168
0.109	8.020	1.345		1.75	k= 3, v=168
0.220	8.020	1.269		1.77	k= 4, v=168

s = 0.357

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	CONTROL	53	0.510	1.370	0.959
2	0.0259	53	0.550	1.400	0.951
3	0.0522	52	0.550	1.360	0.941
4	0.109	8	0.680	1.120	0.858
5	0.220	7	0.560	0.940	0.771

SUMMARY STATISTICS

TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	CONTROL	0.042	0.205	0.028
2	0.0259	0.035	0.186	0.026
3	0.0522	0.042	0.204	0.028
4	0.109	0.030	0.172	0.061
5	0.220	0.014	0.118	0.044

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	53	0.959	0.959	0.959
2	0.0259	53	0.951	0.951	0.951
3	0.0522	52	0.941	0.941	0.941
4	0.109	8	0.858	0.858	0.858
5	0.220	7	0.771	0.771	0.771

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	0.959				
0.0259	0.951	0.214		1.66	k= 1, v=168
0.0522	0.941	0.475		1.73	k= 2, v=168
0.109	0.858	1.377		1.75	k= 3, v=168
0.220	0.771	2.395	*	1.77	k= 4, v=168

s = 0.195

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