

DATA EVALUATION REPORT

1. Chemical: Methidathion
2. Test Material: Supracide® (14C-labeled) (99.2% ai)
3. Study Type: Estuarine Invertebrate Life Cycle
Species Tested: Mysid Shrimp
(Mysidopsis bahia)
same material as used in Fathead FEL # 157350
4. Study ID: Chronic Toxicity of Supracide® to Mysid Shrimp (Mysidopsis bahia). ^{Supplement} Toxicity Test Report No. BW-85-3-1748. August 1985. Conducted by Springborn Bionomics, Inc. Submitted by Ciba-Geigy Corporation. EPA Registration No. 100-530. EPA Accession No. 261874. MRID 157351
5. Reviewed By: John Noles
Biologist
EEB/HED
Signature: *John Noles*
Date: *5/8/87*
6. Approved By: Henry T. Craven
Section Head IV
EEB/HED
Signature: *Henry T. Craven*
Date: *5/18/87*
7. Conclusion:

This study is scientifically sound. The maximum acceptable toxicant concentration (MATC) for mysid shrimp is > 22 < 61 ng/L (parts per trillion).

The study does not fulfill the Guideline requirement, ~~as~~ Supplemental data.
8. Recommendation:

The registrant must submit complete raw data and statistical analysis. EEB needs only the raw data used to conduct the statistical analysis in the study. Also EEB would like a copy of the conducting laboratory's radio-labeling and confirmation procedures.
9. Background:

This study was submitted in response to the Methidathion Registration Standard.
10. Discussion of Individual Tests: N/A.



11. Materials and Methods:

- a. Test Animals - Mysid shrimp, laboratory cultured; 24 hours old; diet - Artemia salina nauplii.
- b. Test System - Intermittent-flow seawater system using a proportional diluter; each aquarium with 9 liters of control seawater or test material; diluter cycle - 7.0 per hr; with 19 volume replacements per aquarium every 24 hours; salinity 31 ppt; temperature - 25 °C.
- c. Dose/Design - 40 shrimp per treatment; measured concentrations: 61 and 83 ng/L (parts per trillion) plus a seawater control and a solvent (DMF) control.

Targeted nominal concentrations: 9.4, 19, 38, 75, and 150 ng/L.

- d. Statistics - ANOVA for evaluating differences between treatment groups. Williams' method (1971) used to evaluate the results in the control and each treatment groups.

12. Reported Results:

[Excerpted from Report]

Results and Discussion

Water quality

During the 28-day test, salinity ranged from 28-31‰ and temperature ranged from 22.4-25.9°C; the mean salinity and temperature were $30 \pm 1\%$ and 24.9 ± 0.9 °C, respectively. The pH ranged from 8.0-8.1. Dissolved oxygen concentrations ranged from 4.8-7.1 mg/L ($> 80\%$ of saturation). Throughout the test these parameters remained within limits considered to be biologically acceptable to mysid shrimp.

Exposure monitoring

During the mysid shrimp chronic test, the mean measured Supracide® concentrations ranged from 6.2 to 83 ng/L in the five treatments. Mean measured concentrations throughout the 28-day test period were between 34 and 81% of the nominal concentrations to be delivered to test aquaria (Tables 1 and 2). There was reasonably good corroboration of measured concentrations between duplicate aquaria at any one sampling interval, but some large variability occurred in some treatments during the 28-day exposure. For example, the Supracide® concentrations measured in the 19 ng/L nominal treatment ranged from 0.9 to 12.2 ng/L (replicate A) and 0.8

to 14.9 ng/L (replicate B), thus resulting in a 28-day mean measured concentration of 6.4 ng/L for this treatment. As a result, the mean measured Supracide® concentration of the lowest (6.2 ng/L) and the second lowest (6.4 ng/L) treatments were the same. Analysis of quality control blind samples at each sampling interval (Table 3) demonstrated satisfactory instrumental and analytical performance (average concentration of $86 \pm 7\%$ of amount fortified was recovered) through test day 21. Analysis of the day 28 quality control blind sample revealed a measured Supracide® concentration of 45% of the amount added to the sample. Overall recovery of Supracide® from fortified blind samples was $79 \pm 18\%$ (Table 3).

Biological observations

Mortality of parental mysids exposed to Supracide® for 28 days ranged from 8% at a mean measured concentration of 6.4 ng/L to 50% at the 83 ng/L test concentration. Mortality in the negative control was 2% (1 mysid of 40) and in the solvent control was 12% (5 mysids of 40). Mortality of parental mysids was statistically significant as compared to the control at Supracide® concentrations of 61 and 83 ng/L. Mysid mortalities at Supracide® concentrations ≤ 22 ng/L were not significantly different from the control (Table 4).

The mean number of offspring (F₁) produced per female (parental) reproductive day ranged from 0.26 in the 83 ng/L Supracide® treatment to 0.50 at 22 n/L Supracide®. The number of total female reproductive days in replicate C of the 22 ng/L Supracide® treatment was inordinately low (3 days) as compared with other replicates (A = 49 days, B = 72 days, D = 43 days) of that treatment. Therefore, data from replicate C were not used in calculating the mean production for this treatment. Production in the control was 0.38 and in the solvent control, 0.43. There were no statistically significant differences in young production between the Supracide® treatments and the control (Table 5).

Mean time to release of first brood was nearly identical (17 to 18 days) in all but one Supracide® test concentration and the controls. At the 6.2 ng/L Supracide® concentration, time to release of first brood was 21 days which was significantly greater than for the control (Table 6).

After 4 days of post-release exposure, mortality of F₁ juvenile mysids ranged from 0% in Supracide® concentrations of 6.4 and 61 ng/L to 15% in 83 ng/L. Mortality of F₁ mysids in the control was 0% and in the solvent control, 5% (Table 7).

Average lengths of parental mysid shrimp after 28 days of exposure to Supracide® ranged from 5.72-5.91 mm for all treatments. Average length of the control population was 5.85 mm and of the solvent control population, 5.78 mm. There were no statistically significant differences between the lengths of mysids from any Supracide® treatment and the control (Table 8.).

The maximum acceptable toxicant concentration (MATC) of Supracide® based on the statistically significant mortality of parental mysids is $>22<61$ ng/L. None of the other criteria considered in evaluating the effects of Supracide® on mysid shrimp demonstrated statistically significant adverse effects due to the 28-day chronic exposure.

Table 1. Results of Chemical Analyses of Supracide[®] in Seawater During A
28-Day Life Cycle Toxicity Test with Mysid Shrimp (*Mysidopsis bahia*)

Test Day	Control		Sol. Control		Measured Concentration (ng/L; pptr)											
	A	B	A	B	9.4				19				38			
					A	B	A	B	A	B	A	B	A	B	A	B
0	ND	ND	ND	ND	5.2	5.0	7.0	10.8	21.7	20.2	60.5	66.0	--	--	73.0	
3	0.7	ND	ND	0.4	6.2	7.1	12.2	14.9	27.5	26.4	57.4	56.8	110.1	110.6		
7	ND	ND	ND	ND	3.8	4.3	7.2	8.1	16.0	15.2	48.7	49.4	54.4	51.5		
14	ND	ND	ND	ND	6.5	7.3	2.2	2.1	29.5	28.1	80.1	80.1	98.9	95.8		
21	ND	ND	ND	ND	7.1	7.8	0.9	0.8	27.9	25.8	66.0	68.2	98.4	102.1		
28	ND	ND	ND	ND	3.9	9.6	6.6	4.6	11.0	9.8	49.7	47.4	65.0	55.6		

^aND = not detected (detection limit 0.3 ng/L; pptr).

Table 2. Mean Measured Concentrations of Supracide® in Seawater During a 28-Day Life-Cycle Toxicity Test with Mysid Shrimp (Mysidopsis bahia).

Nominal Concentration (ng/L; pptr)	Mean Measured Concentration (+ S.D.; ng/L, pptr)		
	Replicate Mean	Treatment Mean	% of Nominal
Control A	ND ^a ^b	ND	--
B	ND		
Solvent Control A	ND	ND	--
B	ND ^b		
9.4 A	5.4 (1.4) ^c	6.2 (1.8)	66
B	6.2 (1.8)		
19 A	6.0 (4.0)	6.4 (4.6)	34
B	6.9 (5.4)		
38 A	22.3 (7.4)	22 (7.0)	57
B	20.9 (7.2)		
75 A	60.4 (11.7)	61 (12)	81
B	61.3 (12.5)		
150 A	85.2 (24.2)	83 (23)	55
B	81.4 (25.)		

^aND = Not detected (detection limit 0.3 ng/L; pptr).

^bOn day 3 of exposure apparent concentrations of 0.7 ng/L and 0.4 ng/L were detected in water samples from control replicate A and solvent control replicate B, respectively.

^cNumbers in parentheses represent standard deviations.

Table 3. Quality Control Sample Results of Supracide® in Seawater Prepared as Blind Samples During a 28-Day Life-Cycle Toxicity Test Using Mysid Shrimp (Mysidopsis bahia)

Test Day	Spiked Concentration (ng/L; pptr)	Measured Concentration (ng/L; pptr)	% of Nominal
0	101	91.7	91
3	40	36.7	92
-	81	71.6	88
14	40	32.8	82
21	81	61.4	76
28	121	54.2	45

$$\bar{x} (\pm \text{S.D.}) = 79 (\pm 18)$$

Table 4. Results of Exposure of Mysid Shrimp (*Mysidopsis bahia*) to Supracide® for 28 Days in an Intermittent-Flow System

Test Day	Cumulative Mortality as Number (Percent) Dead						
	Control	Solvent Control	6.2	6.4	22	61	83
1	0	0	0	0	0	0	0
2	0	1(2)	0	0	0	0	0
3	0	1(2)	0	0	0	1(2)	2(5)
4	0	2(5)	0	1(2)	0	1(2)	3(8)
5	0	2(5)	0	1(2)	0	2(5)	3(8)
6	0	3(8)	0	1(2)	0	2(5)	3(8)
7	0	3(8)	0	1(2)	0	2(5)	3(8)
8	0	3(8)	0	1(2)	0	2(5)	3(8)
9	0	3(8)	0	1(2)	0	2(5)	3(8)
10	0	3(8)	0	1(2)	0	2(5)	3(8)
11	0	3(8)	0	1(2)	0	2(5)	3(8)
12	0	3(8)	0	1(2)	0	2(5)	3(8)
13	0	3(8)	0	1(2)	0	2(5)	3(8)
14	0	3(8)	0	1(2)	1(2)	2(5)	3(8)
15	0	3(8)	0	1(2)	1(2)	2(5)	4(10)
16	0	3(8)	0	1(2)	1(2)	2(5)	4(10)
17	0	3(8)	0	1(2)	2(5)	2(5)	4(10)
18	0	3(8)	1(2)	1(2)	4(10)	3(8)	4(10)
19	0	4(10)	1(2)	1(2)	5(12)	5(12)	5(12)
20	0	4(10)	1(2)	1(2)	8(20)	7(18)	7(18)
21	0	4(10)	1(2)	1(2)	9(22)	9(22)	8(20)
22	0	4(10)	1(2)	1(2)	10(25)	10(25)	9(22)
23	0	4(10)	2(5)	1(2)	10(25)	11(28)	11(28)
24	0	4(10)	2(5)	1(2)	11(28)	11(28)	15(38)
25	0	4(10)	2(5)	2(5)	11(28)	12(30)	16(40)
26	0	4(10)	2(5)	2(5)	11(28)	12(30)	17(42)
27	1(2)	5(12)	2(5)	3(8)	11(28)	12(30)	20(50)
28	1(2)	5(12)	4(10)	3(8)	11(28)	13(32) ^a	20(50) ^a

^aSignificantly ($P \leq 0.05$) greater than the control.

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Table 5. Number of Offspring Per Female Reproductive Day of Mysid Shrimp (Mysidopsis bahia) Exposed to Supracide® in a Chronic (28-Day) Exposure in Natural, Flowing Seawater

Mean Measured Concentration (ng/L; pptr)	Total Offspring/Total Female Reproductive Days					Mean Number of Offspring/Female Reproductive Day
	A	B	C	D	Total Treatment	
Control	19/60	9/36	21/60	43/70	99/226	0.38 (0.16)
← Solvent Control	44/84	16/39	23/72	17/36	100/231	0.43 (0.09)
6.2	35/60	9/24	10/60	16/48	70/192	0.36 (0.17)
6.4	26/68	27/60	19/48	19/36	91/212	0.44 (0.07)
22	30/49	31/72	0/3	20/43	81/167	0.50 ^a (0.10)
61	11/60	26/42	26/28	6/54	69/184	0.46 (0.39)
83	5/33	12/40	9/35	22/66	48/174	0.26 (0.08)

^aData for replicate C, 22 ng/L treatment, were excluded from calculation of the mean for that treatment due to the inordinately low number of total female reproductive days.

Table 6. Time to Release of First Brood by Mysid Shrimp (Mysidopsis bahia) Exposed to Supracide® in a Chronic (28-Day) Exposure in Natural, Flowing Seawater

Mean Measured Concentration (ng/L; pptr)	Test Day of First Brood Release				
	Replicate				Mean of Treatment
	A	B	C	D	
Control	21	17	17	18	18
Solvent Control	17	18	17	17	17
6.2	17	23	24	20	21 ^b
6.4	17	17	17	22	18
22	17	17	— ^a	20	18
61	17	17	17	19	18
83	17	17	17	17	17

^aNo offspring produced.

^bSignificantly ($P \leq 0.05$) greater than the control.

Table 7. Cumulative Mortality of F₁ Mysid Shrimp (*Mysidopsis bahia*) During 96 Hours of Exposure to Supracide® in Natural, Flowing Seawater. Total Number of F₁ Mysids Exposed was 20

Mean Measured Concentration (ng/L; pptr)	Chamber ID	N ^a	Cumulative Mortality (%)				Treatment
			24-HR	48-HR	72-HR	96-HR	
Control	C ₁	5	0	0	0	0	0
	C ₂	8	0	0	0	0	
	C ₃	6	0	0	0	0	
	C ₄	1	0	0	0	0	
Solvent Control	SC ₁	10	0	0	0	0	5
	SC ₂	9	0	11	11	11	
	SC ₃	1	0	0	0	0	
6.2	9.4 ₁	7	0	0	0	0	10
	9.4 ₂	9	11	11	11	11	
	9.4 ₃	4	25	25	25	25	
6.4	19 ₁	10	0	0	0	0	0
	19 ₂	4	0	0	0	0	
	19 ₃	6	0	0	0	0	
22	38 ₁	10	0	0	0	0	5
	38 ₂	6	0	17	17	17	
	38 ₃	4	0	0	0	0	
61	75 _{1A}	10	0	0	0	0	0
	75 _{1B}	10	0	0	0	0	
83	150 ₁	10	10	10	20	20	15
	150 ₂	1	0	0	0	0	
	150 ₃	2	0	0	0	50	
	150 ₄	4	0	0	0	0	
	150 ₅	3	0	0	0	0	

^aN = number of F₁ mysids collected and pooled from screened chambers containing parental mysids on a given test day. Ideally at least 10 mysids could be collected from a given treatment on a test day and placed in a screened chamber in that treatment for testing. However, if less than 10 mysids per day per treatment were collected, mysids would be isolated daily until a total of 20 was obtained.

Table 8. Size of Parental Mysid Shrimp (Mysidopsis bahia) After a Chronic (28-Day) Exposure to Supracide® in Natural, Flowing Seawater

Mean Measured Concentration (ng/L; pptr)	Average Length (mm)
Control	5.85
Solvent Control	5.78
6.2	5.72
6.4	5.91
22	5.78
61	5.84
83	5.81

13. Study Author's Conclusions/QA Measures:

MATC = > 22 < 61 ng/L.

The report certified that EPA GLP was utilized.

14. Reviewer's Discussion and Interpretation of the Study:

- a. Test Procedures - The study was conducted according to acceptable protocol. The following items were observed to be inadequately reported.
 - 1) The complete raw data and statistical analysis were omitted from the submission. EEB requires the raw data and statistical analysis in order to verify the reported results and to make a complete evaluation for validation purposes.
 - 2) EEB would like to review the laboratory's radiolabeling and confirmation procedures, for background information.
- b. Statistical Analysis - EEB cannot conduct a statistical analysis because of the lack of raw data and statistical analysis.
- c. Discussion/Results - Additional information/data are required for further evaluation.

d. Adequacy of Study

- 1) Classification - Supplemental.
- 2) Rationale - Inadequate reporting.
- 3) Reparability - Additional information required for study upgrade considerations.

15. Completion of One-Liner for Study:

One-liner form completed 5/11/87.

16. CBI Appendix: N/A.