

10-11-88

DATA EVALUATION RECORD

PAGE 1 OF

CASE: GS0103

PHORATE FRSTR

CONT-CAT: 01

GUIDELINES: 72-2

MRID: 161825

Nicholson, R.; McNabb, T. (1986) Acute Toxicity of Thimet 20G to Daphnids (*Daphnia magna*): Report #BW-86-6-2055; Study #451.1285. 6108.110. Unpublished study prepared by Springborn Bionomics, Inc. 32 p.

REVIEW RESULTS:

VALID ☒

INVALID ☐

INCOMPLETE ☐

GUIDELINE:

SATISFIED ☒

PARTIALLY SATISFIED ☐

NOT SATISFIED ☐

DIRECT RVW TIME =

2 hr

START DATE:

6/15/88

END DATE:

6/15/88

REVIEWED BY:

Ann Stavola

TITLE:

Aquatic Biologist

ORG:

HED/EEB

LOC/TEL:

CM2 B2D 357 1354

SIGNATURE:

Ann Stavola

DATE:

6/15/88

APPROVED BY:

TITLE:

ORG:

LOC/TEL:

SIGNATURE:

Douglas J. Urban

DATE:

10/11/88

1/3

DATA EVALUATION RECORD

1. Chemical: Phorate

2. Test Material: Thimet 20G, 20% ai

3. Study Type: Freshwater Invertebrate Acute Toxicity

Species Tested: Daphnids
(Daphnia magna)

4. Study ID: MRID No. 161825. Nicholson, R.; McNabb, T. (1986)
Acute Toxicity of Thimet 20G to Daphnids (Daphnia magna): Report No.: BW-86-6-2055, Study No.:
451.1285.6108.110. Unpublished study prepared by
Springborn Bionomics, Inc.

5. Reviewed By: Ann Stavola
Aquatic Biologist
EEB/HED

Signature: *Ann Stavola*

Date: *18 July 88*

6. Approved By: Douglas Urban
Supervisory Biologist
EEB/HED

Signature: *Douglas Urban*

Date: *10/11/88*

7. Conclusion:

This study is scientifically sound and with a 48-hour EC₅₀ value of 37 (30 to 44) ug/L, Thimet 20G is very highly toxic to daphnia magna. This study meets EPA Guidelines requirements for an acute toxicity test on a freshwater invertebrate with a granular formulation of phorate, up to and including 20 percent ai.

8. Recommendations: N/A

9. Background:

Formulated product testing of freshwater invertebrates was required in the Phorate Registration Standard, 1983.

10. Discussion of Individual Tests: N/A

11. Material and Methods:

- a. Test Animals - Species: Daphnids (*Daphnia magna*); Age: < 24 hours at start of study; Source: Bionomics culture facility.
- b. Dosage - Nominal concentrations: 300, 180, 108, 72, and 42 ug/L as Thimet 20G plus control and solvent control. Mean measured concentrations: 48, 22, 14, 8.7, and 2.3 ug/L as phorate. Dilution water: fortified well water, pH 8.0, hard quality, filtered to remove potential organic contaminants. Stock solution: 0.03 g Thimet 20G dissolved in 50 mL acetone; ultrasonicated for 3 hours and supernatant used as stock solution.
- c. Study Design - Protocol closely follows "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (ASTM, 1980). Types of Test Chambers: 250 mL glass beakers containing 200 mL of test solution. Number of Organisms per Test Concentration: 20 daphnids with five daphnids in each of four replicate beakers per concentration. Photoperiod: 16 L:8D; Test Temperature: 20 °C.
- d. Statistical Analyses - Mean measured concentrations (0 and 48 hours) tested and the corresponding mortality data were used to estimate the EC₅₀ values and 95 percent confidence intervals with a computer program (Stephan, 1982). EC₅₀ was defined as the concentration that caused irreversible immobilization of 50 percent of the test population.

12. Reported Results:

Table 1 presents the concentrations measured throughout the study.

Table 1. Results of the analysis of the test solutions during the 48-hour static exposure of daphnids (*Daphnia magna*) to Thimet 20G. Measured concentrations are based on the analyses for Phorate, the active ingredient (20%) of Thimet 20G.

Nominal Concentration (ug/L) As Thimet 20G	Measured Concentration as Phorate (ug/L)			Extrapolated Concentration as Thimet 20G (ug/L)
	0-Hour ^a	48-Hour ^b	Mean (SD) ^c	
Control	< 1.4	< 1.6	< 1.6	< 8.0
Solvent Control	< 1.4	< 1.6	< 1.6	< 8.0
42	4.9	2.7	2.3 (1.9)	12
72	11	6.3	8.6 (3.4)	44
108	18	9.8	14 (5.8)	70
180	31	13	22 (13)	110
300	66	30	48 (25)	240

^aAnalysis of freshly prepared solutions.

^bAnalysis of 48-hour-old solutions.

^cMean (standard deviation) based on the analysis of freshly prepared and aged (48-hour-old) solutions.

Analysis of QA samples showed satisfactory analytical control with a recovery ranging from 95 to 102 percent.

Table 2 presents the cumulative mortality data.

Table 2. Concentrations tested and corresponding cumulative number of affected organisms made during the 48-hour exposure of daphnids (*Daphnia magna*) to Thimet 20G. Concentrations are based on extrapolated mean measured Phorate concentrations.

Extrapolated Mean Measured Concentration (ug/L) as Thimet 20G	Cumulative Number of Affected Organisms (%)									
	24-Hour					48-Hour				
	A	B	C	D	Mean	A	B	C	D	Mean
Control	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	20	0	20	0	10 ^a
44	0	0	60	20	20 ^a	40	40	80	40	50 ^a
70	100	100	100	100	100	100	100	100	100	100
110	100	100	100	100	100	100	100	100	100	100
240	100	100	100	100	100	100	100	100	100	100

^aSeveral of the surviving daphnids were at the bottom of the test vessel.

The calculated LC₅₀ values are:

Time	LC ₅₀ and 95% CI (ug/L)
24-hour	50 (44 to 70)
48-hour	31 (22 to 40)

Dissolved oxygen (DO) and pH were measured at 0 and 48 hours for all concentrations. The pH values were 7.9 to 8.0 at 0 hour and 8.2 at 48 hours. The DO levels were 8.1 to 8.8 ppm at 0 hour and 8.1 to 8.7 ppm at 48 hours. Temperature ranged from 20 to 21 °C.

13. Study Authors' Conclusions/QA Measures:

The 48-hour EC₅₀ value for daphnia magna exposed to Thimet 20G was 31 (22 to 40) ug/L. This indicates Thimet 20G is very highly toxic to daphnids. The NOEL was < 12 ug/L, the lowest concentration tested.

QA Statement: "The data contained in this report were audited by the Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations. . . ."

14. Reviewer's Discussion and Interpretation of Study:

- a. Test Procedures - The test procedures were done in accordance with protocols recommended by EPA Guidelines and are acceptable.
- b. Statistical Analysis - The EC₅₀ values were recalculated with EEB's program, which is also based on a program developed by Stephan.
- c. Results and Discussion - There appears to be an error in Table 1, the calculation of the mean measured concentration for the nominal level 42 ug/L. The mean and S.D. of 4.9 and 2.7 ug/L is 3.8 (1.6) ug/L, and this converts to 19 ug Thimet 20G/L. We used this value (19 ug/L) instead of 12 ug/L, as they reported, in our EC₅₀ calculations.

Our EC₅₀ values were:

<u>Time</u>	<u>EC₅₀ and 95% CI (ug/L)</u>
24-hour	51 (44 to 70)
48-hour	37 (30 to 44)

The NOEL is, therefore, < 19 ug/L.

The data indicates that Thimet 20G is very highly toxic to daphnids.

d. Adequacy of Study

- 1) Classification - Core for granular formulations of phorate up to 20 percent ai.
- 2) Rationale - Scientifically sound study that followed EPA Guidelines. Formulated product testing was required.
- 3) Reparability - N/A

15. Completion of One-Liner for Study: N/A

16. CBI Appendix: N/A

STAVOLA PHORATE DAPHNIA MAGNA 06-15-88

24-hr

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
240	20	20	100	9.536742E-05
110	20	20	100	9.536742E-05
70	20	20	100	9.536742E-05
44	20	4	20	.5908966
19	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 44 AND 70 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS. BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 50.83267

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

7

STAVOLA PHORATE DAPHNIA MAGNA 06-15-68 *48 hr*

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
240	20	20	100	9.536742E-05
110	20	20	100	9.536742E-05
70	20	20	100	9.536742E-05
44	20	10	50	58.80985
19	20	2	10	2.012253E-02

THE BINOMIAL TEST SHOWS THAT 19 AND 70 CAN BE
 USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
 CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
 ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 44

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
2	7.602794E-02	37.32456	31.51588

43.69267

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1302706	1	.2234498

SLOPE = 5.443261
 95 PERCENT CONFIDENCE LIMITS = 3.478624 AND 7.407898

LC50 = 37.23681
 95 PERCENT CONFIDENCE LIMITS = 30.07499 AND 44.26337

LC10 = 21.75988
 95 PERCENT CONFIDENCE LIMITS = 14.07287 AND 27.50056

8