10-11-88

DATA EVALUATION RECORD PAGE 1 OF

CASE: GS0103 PHORATE FRSTR

CONT-CAT: 01 GUIDELINES: 72-1

MRID: 161823

Nicholson, R.; McNabb, T. (1986) Acute Toxicity of Thimet 20G to Bluegill (Lepomis macrochirus): Report #BW-86-6-2052; Study #451.1285.6108.100. Unpublished study prepared by Springborn Bionomics, Inc. 33 p.

REVIEW RESULTS: VALID ✓ INVALID_____ INCOMPLETE_____

GUIDELINE: SATISFIED ✓ PARTIALLY SATISFIED _____ NOT SATISFIED___

DIRECT RVW TIME = 3 START DATE: 6/14/88 END DATE: 6/14/88

REVIEWED BY: Ann Stavola

TITLE: Aquatic Biologist

ORG: HED/EEB

LOC/TEL: CM2 607D 557 1354

SIGNATURE: Ann Stavola DATE: 6/14/88

APPROVED BY:

TITLE:

ORG:

LOC/TEL:

SIGNATURE: Douglas J. Urban DATE: 10/11/88

DATA EVALUATION RECORD

1. Chemical: Phorate

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

3. Study Type: Freshwater Fish Static Acute Toxicity

   Species Tested: Bluegill (Lepomis macrochirus)

4. Study ID: MRID No. 161823. Nicholson, R.; McNabb, T. (1986) Acute Toxicity of Thimet 20G to Bluegill (Lepomis macrochirus): Report No.: BW-86-6-2052, Study No.: 451.1285.6108.100. Unpublished study prepared by Springborn Bionomics, Inc.

5. Reviewed By: Ann Stavola
   Aquatic Biologist
   EEB/HED
   Signature: Ann Stavola
   Date: 10/11/88

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 96-hour LC_{50} value of 12 (8 to 13) ug/L, Thimet 20G is very highly toxic to bluegill. This study meets EPA Guidelines requirements for an acute toxicity test on a warmwater fish with a granular formulation of phorate, up to and including 20 percent ai.

8. Recommendations: N/A

9. Background:

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

10. Discussion of Individual Tests: N/A

11. Material and Methods:

a. Test Animals - Species: Bluegill (Lepomis macrochirus): Total length: mean = 30 mm: range = 25 to 37 mm: Wet weight: mean = 0.26 g: range = 0.10 to 0.48 g: Source: a commercial supplier; Test material: granular phorate - Thimet 20G; 20 percent ai.

b. Dosage - Nominal concentrations: 100, 60, 36, 22, 13, and 8.0 ug/L as Thimet 20G. Also control and solvent control. Mean measured concentrations: 21, 14, 7.1, 4.7, 3.0, and 2.2 ug/L as the ai phorate and converted to ug of Thimet 20G per liter. Dilution water: deionized reconstituted soft quality well water, pH 7.6. Stock solution: 0.02 g Thimet 20G diluted with 100 mL acetone: ultrasonicated for 3 hours and supernatant used as stock solution.

c. Study Design - Protocol closely followed "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (ASTM, 1980). The test procedure was modified to provide complete solution renewals at 48 hours of the exposure period. This was done to maintain consistent exposure concentrations of the test material. At 48 hours all surviving fish were transferred to a duplicate set of aquaria containing freshly prepared solutions. Types of Test Chambers: 18.9 L glass aquaria holding 15 L of test solution. Number of Organisms per Test Concentration: 10 bluegill were placed in each aquarium. Loading factor: 0.17 g biomass/L test solution. Photoperiod: 16L:8D: Test Temperature: 23 °C.

d. Statistical Analyses - The mean measured concentrations (0 and 48 hours) tested and the corresponding mortality data were used to estimate the LC_{50} values and 95 percent confidence intervals with a computer program (Stephan, 1982).

12. Reported Results:

The stability of Thimet 20G in freshwater was evaluated prior to conducting this study. The half-life of phorate was 97.7 hours. Therefore a 48-hour static renewal procedure was used for this test.

Table 1 presents the concentrations measured throughout the study.

Table 1. Results of the analysis of the test solutions during the 96-hour static exposure of bluegill (Lepomis macrochirus) to Thimet 20G. Test solutions were renewed at 48 hours. 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) 0-Hour[a]	48-Hour[a]	96-Hour[b]	Mean (SD)[c]	Extrapolated Concentration as Thimet 20G (ug/L)
Control	< 0.28	< 0.25	---	< 0.28	< 1.4
Solvent Control	< 0.28	< 0.25	---	< 0.28	< 1.4
8.0	1.7	2.8	---	2.24 (0.80)	11
13	2.7	3.3	---	2.98 (0.45)	15
22	4.6	4.9	---	4.74 (0.18)	24
36	7.1	---d	---	7.1 (0)	35
60	14	---d	---	14 (0)	70
100	21	---d	---	21 (0)	104

[a]Analysis of freshly prepared solutions.
[b]Samples were apparently contaminated during the sampling process, therefore preventing the analyses of Phorate in the exposure solutions at 96 hours.
[c]Mean (standard deviation) based on the analysis of freshly prepared solutions only.
[d]Due to 100 percent mortality of test organisms by 48 hours, this solution was not renewed.

Analysis of QA samples showed satisfactory analytical control with a recovery range of 87 to 112 percent.

Table 2 presents the cumulative mortality data and observations of symptomatic behavior in fish.

Table 2. Concentrations tested and corresponding cumulative mortalities of bluegill (Lepomis macrochirus) exposed to Thimet 20G for 24, 48, 72, and 96 hours. Concentrations are based on extrapolated mean measured Phorate concentrations.

Extrapolated Mean Measured Concentration (ug/L) as Thimet 20G	Cumulative Mortality (%)			
	24-Hour	48-Hour	72-Hour	96-Hour
Control	0	0	0	0
Solvent Control	0	0	0	0
11	0	10[d,f]	40[e]	40[e]
15	0[a]	70[g]	80	90
24	20[b]	90[e,g]	100	100
35	80[c]	100	100	100
70	100	100	100	100
104	100	100	100	100

[a]One fish exhibited a complete loss of equilibrium; one fish exhibited a darkened pigmentation.
[b]Several of the surviving fish exhibited a complete loss of equilibrium and were lethargic.
[c]All of the surviving fish exhibited darkened pigmentation.
[d]Several of the surviving fish exhibited pectoral fins anteriorly extended.
[e]One of the surviving fish was at the surface of the test solution.
[f]One fish was lethargic.
[g]One of the surviving fish exhibited pectoral fins anteriorly extended.

The calculated LC_{50} values are:

Time	LC_{50} and 95% CI (ug/L)
24-hour	29 (24 to 35)
48-hour	14 (12 to 18)
72-hour	12 (7 to 14)
96-hour	12 (7.8 to 13)

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Dissolved oxygen (DO) and pH were measured at 24-hour intervals for each test concentration that still had surviving fish. The pH values ranged from 7.6 and 7.7 at 0-hour and to 6.7 through 7.1 at 96 hours. DO was supersaturated at 0-hour (106 to 108 percent) as the levels were 9.2 to 9.4 ppm. At the end of the first 48-hour period they were reduced to 4.9 to 6.7 ppm, but they were somewhat higher at the end of the second 48-hour period, 5.9 to 8.3 ppm. Temperature was maintained at 23 °C.

13. Study Authors' Conclusions/QA Measures:

The 96-hour LC_{50} for bluegill exposed to Thimet 20G was 12 (7.8 to 13) ug/L. This indicates that Thimet 20G is very highly toxic to bluegill. The NOEL was < 11 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 procedures were done in accordance with protocols recommended by the guidelines. One discrepancy with established protocols is the fish were fed daily during the acclimation period, including the 48-hour prior to beginning the test. The reason for ceasing feed for 48-hour is to prevent the accumulation of uneaten food or fecal material in the test chambers. However since the fish were placed into the test vessels after the test material was added, it is unlikely that there was any significant amounts of organic matter in the chambers. We accept the test procedures.

b. Statistical Analysis - The LC_{50} values were recalculated with our program, which is also based on a program developed by Stephan.

c. Results and Discussion - EEB calculated the LC_{50} values as follows:

Time	EC_{50} and 95% CI (ug/L)
24-hour	29 (24 to 35)
48-hour	14.5 (12 to 18)
72-hour	12 (7 to 14)
96-hour	12 (8 to 13)

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As their results agree with ours, we accept their results as valid.

The data indicate that Thimet 20G is very highly toxic to bluegill.

d. Adequacy of Study

1) Classification - Core for granular 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

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STAVOLA PHORATE BLUEGILL 06-14-68 24-hr

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
104	10	10	100	9.765625E-02
70	10	10	100	9.765625E-02
35	10	8	80	5.46875
24	10	2	20	5.46875
15	10	0	0	9.765625E-02
11	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 15 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 28.98275

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	5.959245E-02	31.70892	26.02338	38.66196

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
9	.4498988	1	.9999658

SLOPE = 10.47525

95 PERCENT CONFIDENCE LIMITS = 3.449028 AND 17.50146

LC50 = 29.01107

95 PERCENT CONFIDENCE LIMITS = 24.29255 AND 35.20485

LC10 = 21.94449

95 PERCENT CONFIDENCE LIMITS = 12.07538 AND 25.66786

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STAVOLA PHORATE BLUEGILL 06-14-88 96-hr

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
104	10	10	100	9.765625E-02
70	10	10	100	9.765625E-02
35	10	10	100	9.765625E-02
24	10	9	90	1.074219
15	10	7	70	17.1875
11	10	1	10	1.074219

THE BINOMIAL TEST SHOWS THAT 11 AND 24 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 13.61561

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.2694203	14.28882	11.15909	17.13977

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.3310052	1	.7367567

SLOPE = 7.523071
95 PERCENT CONFIDENCE LIMITS = 3.194819 AND 11.85132

LC50 = 14.50406
95 PERCENT CONFIDENCE LIMITS = 11.77793 AND 17.57908

LC10 = 9.832757
95 PERCENT CONFIDENCE LIMITS = 5.387437 AND 12.02273

STAVOLA PHORATE BLUEGILL 06-14-88 72-hr

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
104	10	10	100	9.765625E-02
70	10	10	100	9.765625E-02
35	10	10	100	9.765625E-02
24	10	10	100	9.765625E-02
15	10	8	80	5.46875
11	10	4	40	37.69531

THE BINOMIAL TEST SHOWS THAT 0 AND 24 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 11.85084

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	1.252149	11.85084	0	16.13004

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
9	.6764934	1	.9995984

SLOPE = 8.753188
95 PERCENT CONFIDENCE LIMITS = 1.55376 AND 15.95262

LC50 = 11.84158
95 PERCENT CONFIDENCE LIMITS = 7.154145 AND 14.05537

LC10 = 8.478509
95 PERCENT CONFIDENCE LIMITS = 1.20551 AND 10.57417

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96-hr

STAVOLA PHORATE BLUEGILL 06-14-88

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
104	10	10	100	9.765625E-02
70	10	10	100	9.765625E-02
35	10	10	100	9.765625E-02
24	10	10	100	9.765625E-02
15	10	9	90	1.074219
11	10	4	40	37.69531

THE BINOMIAL TEST SHOWS THAT 0 AND 15 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 11.63965

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.7206815	11.63965	6.113809	13.32592

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
24	.7026664	1	.9999997

SLOPE = 11.43644
95 PERCENT CONFIDENCE LIMITS = 1.849822 AND 21.02305

LC50 = 11.57887
95 PERCENT CONFIDENCE LIMITS = 7.532758 AND 13.40033

LC10 = 8.966394
95 PERCENT CONFIDENCE LIMITS = 1.694083 AND 10.6702

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