DATA EVALUATION RECORD

CASE: GS0103
PHOSPHATE FRSTR

CONT-CAT: 01 GUIDELINES: 72-1

MRID: 161822


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:

Finn Staakla
TITLE: Aquatic Biologist
ORG: LEEF
LOC/TEL: CM2 407 D 551 135Y
SIGNATURE: C.W. DEVEAUX
DATE: 6/15/88

APPROVED BY:

Douglas J. Urban
TITLE: Supervisory Biologist
ORG: NED/EB
LOC/TEL: CM2 815H 557-4365
SIGNATURE: Douglas J. Urban
DATE: 8/1/88
1. Chemical: Phorate

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

3. Study Type: Freshwater Fish Static Acute

   Species Tested: Rainbow Trout
   (Salmo gairdneri)


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

   Signature: Ann Stavola
   Date: 10/17/88

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

   Signature: Douglas Urban
   Date: 10/17/88

7. Conclusion:

   This study is scientifically sound and with a 96-hour LC50 value of 45 (37 to 57) ug/L, Thimet 20G is very highly toxic to rainbow trout. This study meets EPA Guidelines requirements for an acute toxicity test on a coldwater 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: Rainbow trout (Salmo gairdneri);
   Total Length: mean = 47 mm; range = 39 to 53 mm; Wet
   Weight: mean = 1.1 g; range = 0.63 to 1.38 g; Source: a
   commercial supplier in California.

b. Dosage - Nominal concentrations: 60, 36, 22, 13, 8,
   4.8 ug/L as Thimet 20G. Also control and solvent control.
   Mean measured concentrations: 11, 7.2, 4.3, 3.8, 1.6,
   and 0.84 ug/L as phorate and converted to ug Thimet 20G
   per liter. Dilution water: deionized reconstituted well
   water, soft quality, pH 7.5. Stock solution: 0.06 g
   Thimet 20G dissolved in 500 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). 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:
   19.6-L glass aquaria containing 15 L of test solution.
   Number of Organisms per Test Concentration: 10 rainbow
   trout were placed in each aquarium. Loading Factor:
   0.73 g biomass/L test solution; Photoperiod: 16L:8D;
   Test Temperature: 13 °C.

d. Statistical Analyses - The mean measured concentrations
   (0 and 48 hours) tested and the corresponding mortality
   data were used to estimate the LC50 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 renewal exposure of rainbow trout (*Salmo gairdneri*) 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.

<table>
<thead>
<tr>
<th>Nominal Concentration (ug/L) As Thimet 20G</th>
<th>Measured Concentration as Phorate (ug/L) 0-Hour&lt;sup&gt;a&lt;/sup&gt;</th>
<th>48-Hour&lt;sup&gt;b&lt;/sup&gt;</th>
<th>96-Hour&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Mean (SD)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Extrapolated Concentration as Thimet 20G (ug/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>&lt; 0.32</td>
<td>&lt; 0.24</td>
<td>&lt; 0.25</td>
<td>&lt; 0.32</td>
<td>&lt; 1.6</td>
</tr>
<tr>
<td>Solvent Control</td>
<td>&lt; 0.32</td>
<td>&lt; 0.24</td>
<td>&lt; 0.25</td>
<td>&lt; 0.32</td>
<td>&lt; 1.6</td>
</tr>
<tr>
<td>4.8</td>
<td>0.84</td>
<td>0.87</td>
<td>&lt; 0.25</td>
<td>0.86 (0.021)</td>
<td>4.2</td>
</tr>
<tr>
<td>8.0</td>
<td>1.5</td>
<td>1.7</td>
<td>0.31</td>
<td>1.6 (0.099)</td>
<td>8.0</td>
</tr>
<tr>
<td>13</td>
<td>2.4</td>
<td>5.1</td>
<td>0.51</td>
<td>3.8 (1.9)</td>
<td>19</td>
</tr>
<tr>
<td>22</td>
<td>4.1</td>
<td>4.5</td>
<td>1.0</td>
<td>4.3 (0.28)</td>
<td>21</td>
</tr>
<tr>
<td>36</td>
<td>6.9</td>
<td>7.6</td>
<td>1.3</td>
<td>7.2 (0.50)</td>
<td>36</td>
</tr>
<tr>
<td>60</td>
<td>11</td>
<td>12</td>
<td>2.3</td>
<td>11 (1.1)</td>
<td>57</td>
</tr>
</tbody>
</table>

<sup>a</sup>Analysis of freshly prepared solutions.
<sup>b</sup>Analysis of 48-hour-old solutions.
<sup>c</sup>Mean (standard deviation) based on the analysis of freshly prepared solutions only.

Analysis of QA samples showed satisfactory analytical control with a recovery range of 105 to 108 percent.

Table 2 presents the cumulative mortality data and observations of symptomatic behavior in fish.
Table 2. Concentrations tested and corresponding cumulative mortalities of rainbow trout (Salmo gairdneri) exposed to Thimet 20G for 24, 48, 72, and 96 hours. Concentrations are based on extrapolated mean measured Phorate concentrations.

<table>
<thead>
<tr>
<th>Extrapolated Mean Measured Concentration (ug/L) as Thimet 20G</th>
<th>Cumulative Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24-Hour</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Solvent Control</td>
<td>0</td>
</tr>
<tr>
<td>4.2</td>
<td>0</td>
</tr>
<tr>
<td>8.0</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>57</td>
<td>0(^{c})</td>
</tr>
</tbody>
</table>

\(^{a}\) One of the surviving fish was lethargic.  
\(^{b}\) Several of the surviving fish were at the surface of the test solution.  
\(^{c}\) Several of the surviving fish exhibited a partial loss of equilibrium.  
\(^{d}\) Several of the surviving fish exhibited a complete loss of equilibrium.  
\(^{e}\) Several of the surviving fish were lethargic.  
\(^{f}\) All of the surviving fish were lethargic.

The calculated LC\(_{50}\) values are:

<table>
<thead>
<tr>
<th>Time</th>
<th>LC(_{50}) and 95% CI (ug/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour</td>
<td>&gt; 57</td>
</tr>
<tr>
<td>48-hour</td>
<td>&gt; 57</td>
</tr>
<tr>
<td>72-hour</td>
<td>&gt; 57</td>
</tr>
<tr>
<td>96-hour</td>
<td>45 (37 to 57)</td>
</tr>
</tbody>
</table>
Dissolved oxygen (DO) and pH were measured at 24-hour intervals for each test concentration. The pH values were 7.1 and 7.2 at 0-hour and 7.0 and 7.1 at 96 hours. DO was 9.4 to 9.6 ppm at 0-hour, but reduced to 2.5 to 4.9 ppm (24 to 42 percent saturation) at the end of the first 48-hour interval. The lowest level was recorded at 57 ug Thimet/L. At the end of 96-hour, DO levels were 3.9 to 5.7 ppm, and, again, the lowest level was at the highest test concentration. Temperature was maintained at 13 °C.

13. Study Authors' Conclusions/QA Measures:

The 96-hour LC₅₀ for rainbow trout exposed to Thimet 20G was 45 (37 to 57) ug/L. This indicates Thimet 20G is very highly toxic to rainbow trout. The NOEL was 21 ug/L.

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 EPA Guidelines and are acceptable.

b. Statistical Analysis - The LC₅₀ value for 96-hours was recalculated with EEB's program, which is also based on a program developed by Stephan.

c. Results and Discussion - EEB calculated the 96-hour LC₅₀ value to be 45 (37 to 57) ug/L. As their result agrees with ours, we accept their value as valid.

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

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
STAYOLA, PHORATE RAINBOW TROUT 06-14-88

<table>
<thead>
<tr>
<th>CONC.</th>
<th>NUMBER EXPOSED</th>
<th>NUMBER DEAD</th>
<th>PERCENT DEAD</th>
<th>BINOMIAL PROB. (PERCENT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>10</td>
<td>8</td>
<td>80</td>
<td>5.46875</td>
</tr>
<tr>
<td>36</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>5.46875</td>
</tr>
<tr>
<td>21</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>9.765625E-02</td>
</tr>
<tr>
<td>19</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>9.765625E-02</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>9.765625E-02</td>
</tr>
<tr>
<td>4.2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>9.765625E-02</td>
</tr>
</tbody>
</table>

The binomial test shows that 0 and +INFINITY 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 45.299

Results calculated using the moving average method

<table>
<thead>
<tr>
<th>SPAN</th>
<th>G</th>
<th>LC50</th>
<th>95 percent confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.2148829</td>
<td>45.299</td>
<td>37.02619 AND 63.535</td>
</tr>
</tbody>
</table>

Results calculated using the probit method

<table>
<thead>
<tr>
<th>ITERATIONS</th>
<th>G</th>
<th>H</th>
<th>goodness of fit probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.4067773</td>
<td>1</td>
<td>0.999881</td>
</tr>
</tbody>
</table>

Slope = 8.700694
95 percent confidence limits = 3.15147 AND 14.24992

LC50 = 45.39221
95 percent confidence limits = 37.1201 AND 57.11326

LC10 = 32.43516
95 percent confidence limits = 17.49643 AND 39.01996

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