CASE: GS0103
PHORATE FRSTR

CONT-CAT: 01 GUIDELINES: 72-2

MRID: 161825


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: USDA EEb
LOC/TEL: USDA FED 557 1354


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 Invertebrate Acute Toxicity

   Species Tested: Daphnids
   (Daphnia magna)

   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   Unpublished study prepared by Springborn Bionomics, Inc.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: [Signature]
   Date: 07/13/86

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

   Signature: [Signature]
   Date: 10/11/86

7. Conclusion:

This study is scientifically sound and with a 48-hour EC_{50}
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 μg/L as Thimet 20G plus control and solvent control. 
      Mean measured concentrations: 48, 22, 14, 8.7, and 
      2.3 μg/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 Concen- 
      tration:** 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.

<table>
<thead>
<tr>
<th>Nominal Concentration (ug/L) As Thimet 20G</th>
<th>Measured Concentration as Phorate (ug/L)</th>
<th>Extrapolated Concentration as Thimet 20G (ug/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>&lt; 1.4 &lt; 1.6 &lt; 1.6</td>
<td>&lt; 8.0</td>
</tr>
<tr>
<td>Solvent Control</td>
<td>&lt; 1.4 &lt; 1.6 &lt; 1.6</td>
<td>&lt; 8.0</td>
</tr>
<tr>
<td>42</td>
<td>4.9 2.7       2.3 (1.9)</td>
<td>12</td>
</tr>
<tr>
<td>72</td>
<td>11 6.3       8.6 (3.4)</td>
<td>44</td>
</tr>
<tr>
<td>108</td>
<td>18 9.8       14 (5.8)</td>
<td>70</td>
</tr>
<tr>
<td>180</td>
<td>31 13       22 (13)</td>
<td>110</td>
</tr>
<tr>
<td>300</td>
<td>66 30       48 (25)</td>
<td>240</td>
</tr>
</tbody>
</table>

\(^a\) Analysis of freshly prepared solutions.
\(^b\) Analysis of 48-hour-old solutions.
\(^c\) Mean (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.

<table>
<thead>
<tr>
<th>Extrapolated Mean Measured Concentration (ug/L) as Thimet 20G</th>
<th>Cumulative Number of Affected Organisms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24-Hour</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Solvent CONTROL</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>110</td>
<td>100</td>
</tr>
<tr>
<td>240</td>
<td>100</td>
</tr>
</tbody>
</table>

*a Several of the surviving daphnids were at the bottom of the test vessel.

The calculated LC<sub>50</sub> values are:

<table>
<thead>
<tr>
<th>Time</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; and 95% CI (ug/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour</td>
<td>50 (44 to 70)</td>
</tr>
<tr>
<td>48-hour</td>
<td>31 (22 to 40)</td>
</tr>
</tbody>
</table>

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 EC<sub>50</sub> 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 EC$_{50}$ values were recalculated with EBB'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 EC$_{50}$ calculations.

Our EC$_{50}$ values were:

<table>
<thead>
<tr>
<th>Time</th>
<th>EC$_{50}$ and 95% CI (ug/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour</td>
<td>51 (44 to 70)</td>
</tr>
<tr>
<td>48-hour</td>
<td>37 (30 to 44)</td>
</tr>
</tbody>
</table>

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
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.
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

<table>
<thead>
<tr>
<th>SPAN</th>
<th>LC50</th>
<th>95 PERCENT CONFIDENCE LIMITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7.602794E-02</td>
<td>37.32456 31.51588</td>
</tr>
</tbody>
</table>

43.69267

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>6</td>
<td>.1302706</td>
<td>.2234498</td>
<td></td>
</tr>
</tbody>
</table>

SLOPE = 5.443261
95 PERCENT CONFIDENCE LIMITS = 3.478624 AND 7.407898

LC50 = 37.23661
95 PERCENT CONFIDENCE LIMITS = 30.07499 AND 44.26337

LC10 = 21.75988
95 PERCENT CONFIDENCE LIMITS = 14.07287 AND 27.50056

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