**EEB BRANCH REVIEW**

**DATE:** IN 4/28/82  OUT 6/7/82

**FILE OR REG. NO.** 241-243

**PETITION OR EXP.**

**DATE OF SUBMISSION** April 13, 1982

**DATE RECEIVED BY HED** April 26, 1982

**RD REQUESTED COMPLETION DATE** August 16, 1982

**EEB ESTIMATED COMPLETION DATE**

**RD ACTION CODE/TYPE OF REVIEW** 400/

**TYPE PRODUCT(S):** I, D, H, F, N, R, S  Herbicide

**DATA ACCESSION NO(S).**

**PRODUCT MANAGER NO.** R. Taylor (25)

**PRODUCT NAME(S)** PROWL

**COMPANY NAME** American Cyanamid Company

**SUBMISSION PURPOSE** Submission of Chronic *Daphnia* Study in Support of Registration.

<table>
<thead>
<tr>
<th>SHAUGHNESSEY NO.</th>
<th>CHEMICAL &amp; FORMULATION</th>
<th>% A.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>108501</td>
<td>(N-(1-etylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine)</td>
<td></td>
</tr>
</tbody>
</table>
100. **Pesticide Label Information**

This submission contains only data requests made in connection with conditional registration. Therefore, no labeling was submitted.

101. **Physical and Chemical Properties**

101.1 **Chemical Name**

(N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine)

101.2 **Structural Formula**

\[
\begin{array}{c}
\text{CH}_2\text{H}_5 \\
\text{CH} \\
\text{O}_2\text{N} \\
\text{NH} \\
\text{NO}_2 \\
\text{C}_2\text{H}_5 \\
\text{CH}_{3} \\
\text{CH}_{3}
\end{array}
\]

101.3 **Common Names**

Penoxy'n, Penoxalin, Pendimethalin, AC92,553 CL92,553

101.4 **Trade Name**

Prowl

101.5 **Molecular Weight**

281.3
101.6 Physical State

Color and State .......... Orange-yellow crystals
Odor ..................... Faint, nutty odor
Boiling point .......... 330°C
Melting point .......... 56-57°C
Specific Gravity ........ 1.9 @ 25°C
Vapor Pressure .......... $3.0 \times 10^{-5}$ mm Hg @ 25°C
Stability ............... Stable to Alkaline and Acidic Conditions
Corrosiveness .......... Non-corrosive

101.7 Solubility

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Temp. °C</th>
<th>Solubility (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>23</td>
<td>0.5</td>
</tr>
<tr>
<td>Acetone</td>
<td>26</td>
<td>699</td>
</tr>
<tr>
<td>Xylene</td>
<td>26</td>
<td>628</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>26</td>
<td>77</td>
</tr>
<tr>
<td>Corn oil</td>
<td>26</td>
<td>148</td>
</tr>
</tbody>
</table>

107. Conclusions

107.4 Data Adequacy Conclusions

The attached study entitled, Chronic (21-Day) toxicity of AC92, 553 to *Daphnia magna* Straus, does not meet guideline requirements in that the grade of the chemical tested was not identified or was the percent of active ingredient given.
Dennis J. McLane, Wildlife Biologist
Ecological Effects Branch
Hazard Evaluation Division (TS-769)

Raymond W. Matheny
Head, Review Section No. 1
Ecological Effects Branch
Hazard Evaluation Division (TS-769)

Clayton Bushong, Chief
Ecological Effects Branch
Hazard Evaluation Division (TS-769)

DATA EVALUATION RECORD

1. CHEMICAL: (N-(1-Ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) [Prowl/Pendimethalin]

2. FORMULATION: Not reported


4. REVIEWED BY: Dennis J. McLane, Wildlife Biologist Ecological Effects Branch Hazard Evaluation Division (TS-769)

5. DATE REVIEWED: May 20, 1982

6. TEST TYPE: Daphnia magna life-cycle toxicity

7. REPORTED RESULTS: The LC50's calculated for 4 and 8 days respectively, were 32.5 (23.6-44.7) and 19.1 (confidence limits unobtainable) ug/l. The 15 and 21 day LC50 values were 18.2 ug/l and 19.0 ug/l, respectively. Both values were corrected to compensate for control mortality. After 15 day and 21 days the 22.1 and 17.2 ug/l levels, respectively, reduced cumulative production by 50% (RI50). The calculated 10, 15 and 21 day no observed effect concentration (NOEC), based on total production was 14.5 ug/l.

8. REVIEWER'S CONCLUSIONS: This study is scientifically sound. However, it does not meet the guideline requirements. The report fails to indicate the percent active ingredient or the grade of the chemical.

\[ \text{Signature}\]

\[ D. \text{McLane}\]

\[ 5/20/82\]
B. Statistical Analysis

The LC50's were determined by the Litchfield and Wilcoxon Method (1949). Abbott's formula was used to adjust the LC50 values to determine the affect of control mortality.

The percent reproductive impairment, R.I.50 was determined by the Litchfield and Wilcoxon Method (1949).

The effect of different treatment levels on Daphnia brood sizes and cumulative young production were determined using Analysis of Variance (ANOVA) and Duncans Multiple Range Test.

The No Observed Effect Concentration (NOEC) was calculated as the highest concentration tested in which the number of young produced did not differ significantly from that in the controls, as determined by Duncans Multiple Range Test.

C. Discussion/Results

1. LC50's, ug/l

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 15</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>C</td>
<td>U</td>
<td>C</td>
</tr>
<tr>
<td>LC50</td>
<td>32.5</td>
<td>19.1</td>
<td>17.2</td>
<td>17.2</td>
</tr>
<tr>
<td>95% Lower</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confidence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limits</td>
<td>Upper</td>
<td>N.O.</td>
<td>15.2</td>
<td>N.O.</td>
</tr>
<tr>
<td></td>
<td>44.7</td>
<td>N.O.</td>
<td>19.4</td>
<td>N.O.</td>
</tr>
</tbody>
</table>

2. RI50: 15 Day RI50 - 22.1 ug/l
   21 Day RI50 - 17.2 ug/l

Effect on Overall Survival:

By day 6, 100% mortality had occurred in the two highest concentrations (35.8 and 74.2 ug/l). At test termination, there was no appreciable mortality at the three lowest exposure levels (4.3, 8.2 and 14.5 ug/l).
3. **Effects on Overall Productivity:**

Duncans Multiple Range Test indicates that cumulative production means for control, solvent control and 3 treatments (4.3, 8.2 and 14.5 ug/l) were not significantly different.

4. **Effect on Mean Brood Sizes:**

Duncans Multiple Range Test indicates that AC 92,553 had no significant effect on mean brood size.

5. **Calculated NOEC:** 14.5 ug/l.

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N.O. - Not obtainable.
U - Uncorrected for control mortality.
C - Corrected for control mortality.

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**Reviewer's Evaluation**

**A. Test Procedures**

The author made the following statements concerning deviations from the protocol:

1. The temperature range was 21°C - 22°C as opposed to protocols stated temperature of 20 ± 1°C.

2. The water acidity was not measured.

3. Analytical determinations of the concentrations were made on days 0, 1, 3, 7, 10, 14, 17 and 21, with each stock solution being analytically verified. The protocol stated that analytical samples were to be frozen and analyzed at the end of the test, with stock solutions being analyzed weekly.

4. Flow rate during the test resulted in, approximately 2.8 turnovers per day as opposed to protocol stated for 4-6 to turnovers.

5. Diluter operation (i.e., test volume delivery, number of cycles, etc.) was checked daily, however, not recorded.
6. The No Observed Effect Concentration (NOEC) was calculated instead of the Maximum Acceptable Toxicant Concentration (MATC); however, for this study they are numerically the same.

In addition to these amendments to the protocol (ASTM Draft No. 3 March 1981) there was no mention of a 21 holding period prior to the study, nor did the report indicate, prior to starting a test, that Daphnia which are at least 10-12 days old (those which have had at least on brood) should be separated from the culture, put in a separate culture container and maintained as described above. Also, the report indicates 5 of the instars were selected and distributed at random to each test vessel, a total of 20 daphnids for each toxicant concentration and control. The protocol indicates that each concentration is replicated 4 times using 10 Daphnia per replicate yielding a total of 40 Daphnia per treatment.

The test material formulation was not given, that is, the chemical grade and percent active ingredient.

B. Statistical Analysis

Four portions of the study required statistical interpretation. First, the LC50 for days 4, 8, 15, and 21 were calculated. These were verified by EEB's Stephan's computer program (see the attached results). The resulting values did not significantly vary from Biospherics.

The next two items require ANOVA interpretations: cumulative young product and mean brood sizes. EEB's SAS ANOVA and Duncans Multiple Range Test are in agreement with Biospherics, that the treatment level of 14.5 ug/l and below are not significantly different from the control. However, it should be mentioned that for the 17-21 day interval for the mean brood size for the 14.5 ug/l level was significantly different from the solvent control, 4.3, and 8.2 ug/l but not the control.

Lastly, the RI50 for 21 days was verified by the Stephan's program (see attached printout). This value 19.9 ug/l (C.L. of 14.5 to 35.8 ug/l for the binomial method) is not significantly different than the reported value of 22.1 ug/l.

Discussion/Results

The study presents a relative comparison of the chronic effects to Daphnia. Though the holding period was not reported, test dates indicated a 21 day period. However, the test material formulation is required.
Conclusion:

1. Category - Supplemental

2. Rationale - The study is scientifically sound. It does not meet the guideline requirements. The study failed to report the formulation (percent of active ingredient or grade of the chemical).

3. Repairability - Yes, provide the formulation for the test material.

**Daphnia LC50 uncorrected for control mortality** (ug/l)

**Day 4**

Binomial LC50 31.99

Moving Average LC50 34.37  C.L. 27.9 - 44.3

Probit LC50 31.29  C.L. 0 - infinity

**Day 8**

Binomial LC50 21.5

Moving Average LC50 17.7

Probit LC50 17.6  C.L. 0 - infinity

**Day 15**

Binomial LC50 19.91  C.L. 14.5 - 35.8

Moving Average LC50 16.2  C.L. 12.8 - 20.62

Probit LC50 16.3  C.L. 0 - infinity

**Day 2**

Binomial LC50 19.2  C.L. 14.5 - 35.8

Moving Average LC50 14.86  C.L. 11.3-19.34

Probit LC50 14.77  C.L. 0 - infinity
<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>35.8</td>
<td>901</td>
<td>901</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>14.5</td>
<td>901</td>
<td>120</td>
<td>13.31853</td>
<td>0</td>
</tr>
<tr>
<td>8.2</td>
<td>901</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.3</td>
<td>901</td>
<td>33</td>
<td>3.662597</td>
<td>0</td>
</tr>
</tbody>
</table>

The binomial test shows that 14.5 and 35.8 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 19.86833.

The moving average method cannot be used with this data set because no span which produces moving average angles that bracket 45 degrees also uses two percent dead between 0 and 100 percent.

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>11</td>
<td>35.69536</td>
<td>2348.07</td>
<td>0</td>
</tr>
</tbody>
</table>

A probability of 0 means that it is less than 0.001.

Since the probability is less than 0.05, results calculated using the probit method probably should not be used.

Slope = 5.370687
95 percent confidence limits = -26.7168 and 37.45818

LC50 = 19.01054
95 percent confidence limits = 0 and +infinity

LC10 = 11.02873
95 percent confidence limits = 0 and +infinity.
NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

MEAN PROWL DAPHNIA LC50

<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>74.2</td>
<td>18</td>
<td>18</td>
<td>100</td>
<td>0.0003814697</td>
</tr>
<tr>
<td>35.8</td>
<td>18</td>
<td>18</td>
<td>100</td>
<td>0.0003814697</td>
</tr>
<tr>
<td>14.5</td>
<td>18</td>
<td>2</td>
<td>11.1111</td>
<td>0.06561279</td>
</tr>
<tr>
<td>8.2</td>
<td>18</td>
<td>1</td>
<td>5.5556</td>
<td>0.007247925</td>
</tr>
<tr>
<td>4.3</td>
<td>18</td>
<td>1</td>
<td>5.5556</td>
<td>0.007247925</td>
</tr>
</tbody>
</table>

THE BINOMIAL TEST SHOWS THAT 14.5 AND 35.8 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 20.49941

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>4</td>
<td>0.0528356</td>
<td>18.4985</td>
<td>14.70402 23.662</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>2.742576</td>
<td>8.212363</td>
<td>0</td>
</tr>
</tbody>
</table>

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.425728
95 PERCENT CONFIDENCE LIMITS = -2.903598 AND 11.75505

LC50 = 18.29986
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 9.451252
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

*****************************************************************************
NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

** PROW L DAPHINIA LC50 **

<table>
<thead>
<tr>
<th>CONC.</th>
<th>NUMBER</th>
<th>NUMBER</th>
<th>PERCENT</th>
<th>BINOMIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>74.2</td>
<td>17</td>
<td>17</td>
<td>100</td>
<td>0.0007629395</td>
</tr>
<tr>
<td>35.8</td>
<td>17</td>
<td>17</td>
<td>100</td>
<td>0.0007629395</td>
</tr>
<tr>
<td>14.5</td>
<td>17</td>
<td>1</td>
<td>5.8824</td>
<td>0.01373291</td>
</tr>
<tr>
<td>8.2</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0.0007629395</td>
</tr>
<tr>
<td>4.3</td>
<td>17</td>
<td>1</td>
<td>5.8824</td>
<td>0.01373291</td>
</tr>
</tbody>
</table>

THE BINOMIAL TEST SHOWS THAT 14.5 AND 35.8 CAN BE USED AS STATISTICALY 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 21.328

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
SPAN G LC50 95 PERCENT CONFIDENCE LIMITS
4 0.05504922 21.07382 16.68025 27.50513

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS G H GOODNESS OF FIT PROBABILITY
8 10.48471 28.37556 0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.810362
95 PERCENT CONFIDENCE LIMITS = -10.76564 AND 20.38636

LC50 = 19.7356
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 10.74575
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

*******************************************************************************
### Methods and Materials

#### A. Test Procedures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measure, Setting or Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Test material</td>
<td>AC 92,553 (Prowl)</td>
</tr>
<tr>
<td>2. Test type</td>
<td>Daphnia magna 21-Day Chronic (flow-through)</td>
</tr>
<tr>
<td>3. Test date</td>
<td>7/16/81 - 9/8/81</td>
</tr>
<tr>
<td>4. Physical test apparatus, toxicant injection system</td>
<td>Ace Glass solenoid diluter with teflon fittings and tubing. Direct flow toxicant delivery system.</td>
</tr>
<tr>
<td>5. Nominal toxicant stock solution concentration.</td>
<td>75.0 μg/l</td>
</tr>
<tr>
<td>6. Cumulative Mean Measured test water concentration</td>
<td>4.3, 8.2, 14.5, 35.8 and 74.2 μg/l plus control and solvent control (0.5 mg/l acetone)</td>
</tr>
<tr>
<td>7. Test vessels</td>
<td>Twenty-eight rectangular battery jars (10 x 10 x 20 cm; 1.3 liter capacity).</td>
</tr>
<tr>
<td>8. Dilution water and volume</td>
<td>Biospherics Incorporated site well water; 20 minute cycle time; 50 ml per replicate per cycle.</td>
</tr>
<tr>
<td>9. Mean water quality characteristics</td>
<td>Total hardness 115 mg/l as CaCO₃, total alkalinity 147 mg/l as CaCO₃, conductivity 352 μhmhos/cm, pH range 7.3 - 7.6.</td>
</tr>
<tr>
<td>10. Photoperiod</td>
<td>Sixteen hours light, eight hours darkness. Light provided by General Electric Gro and Sho fluorescent lights (Color rendering index &gt;90). Surface light intensity 575 - 625 lux.</td>
</tr>
<tr>
<td>11. Bioassay organism</td>
<td>Daphnia magna Straus obtained from laboratory stock culture. Twenty organisms per concentration (5 per replicate).</td>
</tr>
<tr>
<td>12. Feeding rate, food</td>
<td>40 mg/test vessel/day (Trout chow and yeast suspension).</td>
</tr>
<tr>
<td>13. Mortality and productivity counts</td>
<td>Counts made on days 1, 2, 3, 4, 6, 8, 15, 13, 15, 17 and 21. Dead individuals and instars removed on these days.</td>
</tr>
</tbody>
</table>
### General Linear Models Procedure

**Dependent Variable:** Response

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
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<td>128,803,00000</td>
<td>32,200,75000</td>
<td>3.73</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>129,445,00000</td>
<td>8,629,66667</td>
<td>PR &gt; F</td>
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<tr>
<td>Corrected Total</td>
<td>19</td>
<td>258,248,00000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**R-Square:** 0.492757

**C.V.:** 20.3156

**STD DEV:** 2.93762943

**Response Mean:** 14.46000000

<table>
<thead>
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<th>Source</th>
<th>DF</th>
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<th>PR &gt; F</th>
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<td>3.73</td>
<td>0.0267</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type IV SS</th>
<th>F Value</th>
<th>PR &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>4</td>
<td>128,803,00000</td>
<td>3.73</td>
<td>0.0267</td>
</tr>
</tbody>
</table>
11.200000
-12.75000
-16.15000
16.75000
16.800000

GROUPING
MEAN
VARIABLE

PROBABILITY

ALPHA LEVEL = .05
DF = 15
MS = 8.62967

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

GENERAL LINEAR MODELS PROCEDURE

3119 WEDNESDAY, MAY 26, 1982
STATISTICAL ANALYSIS SYSTEMS