DATA EVALUATION RECORD

1. **CHEMICAL:** Tetramethrin  
   Shaughnessey No. 69003

2. **TEST MATERIAL:** Neo-Pynamin; Lot No. 90304; 95.3% active ingredient; a powder.

3. **STUDY TYPE:** Freshwater Invertebrate Acute Flow-Through Toxicity Test. Species Tested: *Daphnia magna*


5. **REVIEWED BY:**  
   Louis M. Rifici, M.S.  
   Associate Scientist II  
   KBN Engineering and Applied Sciences, Inc.  
   Signature: [Signature Image]  
   Date: 2/22/91

6. **APPROVED BY:**  
   Pim Kosalwat, Ph.D.  
   Senior Scientist  
   KBN Engineering and Applied Sciences, Inc.  
   Signature: [Signature Image]  
   Date: 2/22/91

   Henry T. Craven, M.S.  
   Supervisor, EEB/HED  
   USEPA  
   Signature: [Signature Image]  
   Date: 3/20/92

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an acute flow-through toxicity test for freshwater invertebrates. Test concentrations measured at 0 and 48 hours were greatly different. The actual concentrations the daphnids were exposed to are unknown. Based on mean measured concentrations, the 48-hour LC50 was 0.045 mg/L. Therefore, Neo-Pynamin is classified as very highly toxic to *Daphnia magna*. The NOEC was estimated as 0.024 mg/L.

8. **RECOMMENDATIONS:** N/A

[Handwritten Note Image]
9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: *Daphnia magna* were obtained from in-house cultures. The primary culture was obtained from the Columbia National Fisheries Research Laboratory in Columbia, MO. The cultures were housed in a temperature controlled area (20°±2°C) on a 16-hour daylight photoperiod (30 minute dawn/dusk simulation). The light intensity was maintained at 50-70 ft-candles. Blended hard water (well water and reverse-osmosis water) was used to culture the daphnids.

At least every three days, adult daphnids were fed algae (*Selenastrum capricornutum*) and a supplement of a Tetramin®/cereal leaves/yeast suspension.

Less than 24-hour old, first instar daphnids were selected for the test.

B. Test System: A 10,000 mg/L diluter stock solution (corrected for compound purity) was prepared by diluting 1.0493 g Neo-Pyramid to 100 mL in acetone. The stock was delivered to the diluter using a syringe pump. The proportional diluter delivered 3.5 mL/chamber/minute to each of four 1-liter test vessels per concentration (or 5.0 volume replacements per day). The test vessels were glass beakers, 15 cm high and 10.5 cm wide, with notched drains covered by 50-mesh stainless steel screens. The average vessel solution replacement was five volumes of test solution every 24 hours over the course of the study.

The characteristics of the dilution water are given in Table 1 (attached). The test chambers were immersed in a temperature-controlled water bath set to 20°C±1°C. The photoperiod was the same as in culturing with a light intensity of 45 ft-candles.

The daphnids were not fed during the test.

C. Dosage: Forty-eight-hour static test. Based on preliminary tests, five nominal concentrations (0.06, 0.12, 0.25, 0.50, and 1.0 mg/L), a dilution water control, and a solvent control (0.05 mL acetone/L) were used.
D. **Design:** Four chambers were used for each concentration with ten daphnids per chamber. All concentrations were observed at 24 and 48 hours for mortality and abnormal effects such as immobilization, surfacing, clumping together, and lying on the bottom of the chambers. The temperature, dissolved oxygen (D.O.), and pH were measured in the dilution water control, low, middle and high concentrations at the beginning and end of the test.

Neo-Pynamin concentrations were measured by gas-liquid chromatography analysis from samples taken at test initiation and termination.

E. **Statistics:** The 48-hour median lethal concentration (LC$_{50}$) and associated 95% confidence interval (C.I.) were calculated using a computer program developed by Stephan et al. (1978).

12. **REPORTED RESULTS:** The mean measured concentrations were 0.024, 0.038, 0.084, 0.15, and 0.30 mg/L. Measured concentrations at 0 and 48 hours averaged 53 ±8.3% and 13 ±2.3% of nominal, respectively (Table 2, attached).

The responses of *Daphnia magna* are given in Table 5 (attached). The 48-hour LC$_{50}$ based on mean measured concentrations was 0.035 mg/L (95% C.I. = 0.024-0.038 mg/L). The slope of the dose-response curve was given as 5.2. The no-observed-effect concentration (NOEC), based on the lack of mortality and abnormal effects, was 0.024 mg/L after 48 hours.

Dissolved oxygen ranged from 8.2 to 9.2 mg/L or 94 to 106% of saturation. The pH values ranged from 7.8 to 7.9. The temperature was 19° to 20°C throughout the test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The author presented no conclusions.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**
A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated from the SEP as follows:

Oxygen saturation was greater than 100% (the recommended range is 60%-100% at initiation) in some solutions at test initiation (103-106%).

Each selected nominal concentration was approximately 50% of the next highest concentration. The SEP recommends that each concentration be 60% of the next highest concentration.

First instar *Daphnia magna* used in tests should be from the fourth or later broods of a given parent. The author did not indicate which brood was the source of the test animals.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the 48-hour LC₉₀ as 0.045 mg/L (95% C.I. = 0.040-0.050 mg/L) by the moving average method (see attached printout). The report author used the binomial test (to get an LC₉₀ of 0.035 mg/l), but, due to the large number of animals at each level (40), the moving average method is recommended.

C. **Discussion/Results:** Judging from the response of the control organisms, oxygen supersaturation did not modify the response of the daphnids in the test (Table 5, attached).

The loss of Neo-Pynamin may be the result of temperature related decomposition. A memo from the manufacturer to ABC Laboratories in the Appendix of the bluegill study report (MRID No. 416096-07; p. 170) indicated that Neo-Pynamin decomposed under static range-finding conditions. It is unlikely that rapid decomposition would take place under flow-through conditions. However, the three flow-through, acute studies reviewed experienced 29 to 87% reductions from nominal test concentrations. The greatest decreases are found in tests using higher temperatures. The largest decrease (87% reduction) is found at 19°-20°C and 5.0 volume replacements per day in this test. The temperature in the bluegill test was 22°-23°C with 6.8 tank volume replacements per day but the loss of active ingredient averaged only 55%. In any future tests of this product, increasing the number of volume replacements to near ten per day may be helpful in
However, the differences in measured values at the beginning and end of the test are large and suggest that the actual concentrations the daphnids were exposed to are not known. Further, the high mortality rate albeit the rapid test material decomposition suggest degrade toxicity. This leaves open the avenue of repeating the test using a higher turnover rate to maximize the toxicity effects of the active ingredient, or to identify and test the primary degrade.

This study is scientifically sound but does not satisfy the guideline requirements for an acute flow-through toxicity test. Under the conditions of the test, the 48-hour LC₅₀ of 0.045 mg/L (based on mean measured concentrations) classifies Neo-Pyamin as very highly toxic to Daphnia magna. The NOEC can be estimated as 0.024 mg/L.

D. Adequacy of the Study:

(1) Classification: Supplemental

(2) Rationale: The actual concentrations the daphnids were exposed to are unknown. The high mortality rate and the rapid loss of test material suggest degradation product toxicity.

(3) Repairability: None. The study should be repeated using a higher turnover rate that would maximize the concentrations and effects of the measured active ingredient.

CONC. | EXPOSED | NUMBER | DEAD | PERCENT | BINO MIAL | DEAD | PROB. (PERCENT)
------|---------|--------|------|----------|----------|------|----------------
.3    | 40      | 40     | 100  | 0        |
.15   | 40      | 40     | 100  | 0        |
.084  | 40      | 35     | 87.5 | 0        |
.038  | 40      | 27     | 67.5 | 0        |
.024  | 40      | 0      | 0    | 0        |

Because the number of organisms used was so large, the 95 percent confidence intervals calculated from the binomial probability are unreliable. Use the intervals calculated by the other tests.

An approximate LC50 for this set of data is 3.469867E-02

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>3</td>
<td>2.372141E-02</td>
<td>4.476816E-02</td>
</tr>
<tr>
<td></td>
<td>3.971285E-02</td>
<td>4.985092E-02</td>
</tr>
</tbody>
</table>

Results calculated using the probit method

<table>
<thead>
<tr>
<th>ITERATIONS</th>
<th>H</th>
<th>GOODNESS OF FIT PROBABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1.181076</td>
<td>6.076065</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 = 5.04266
95 PERCENT CONFIDENCE LIMITS = -.4375682 AND 10.52289

LC50 = 4.065973E-02
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 0.0227674
95 PERCENT CONFIDENCE LIMITS = 0 AND 3.765956E-02
The material not included contains the following type of information:

___ Identity of product inert ingredients.
___ Identity of product impurities.
___ Description of the product manufacturing process.
___ Description of quality control procedures.
___ Identity of the source of product ingredients.
___ Sales or other commercial/financial information.
___ A draft product label.
___ The product confidential statement of formula.
___ Information about a pending registration action.
___ FIFRA registration data.
___ The document is a duplicate of page(s) ______.
___ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.