FISH TOXICITY LABORATORY REPORT
Animal Biology Laboratory
ARS-PR, ARC, Beltsville, Md.

Test Number: 477

Product: Tordon 225
Manufacturer: Dow Chemical Company
Midland, Michigan

Active Ingredients:
- 4-amino-3,5,6-trichloropicolinic acid as the triethylamine salt 15.2%
- 2,4,5-trichlorophenoxyacetic acid as the triethylamine salt 14.9%

Date Product Received: April 11, 1972

Period of Test: April 25 - 29, 1972

Biologist Conducting Test: John A. McCann

Test Species: Rainbow trout (Salmo gairdneri)
Condition: Excellent
- Average length: 44.1 mm
- Average weight: 0.767 gm

Source: Wytheville National Fish Hatchery
Date received: February 29, 1972

Acclimation temperature: 55 °F

Bioassay Conditions:
- Test vessel: 5-gallon glass jar.
- Fish/vessel: 10
- Fish/concentration: 20
- Water volume: 15 l
- Concentrations tested: 6

Water Quality:
- Test Water: Demineralized water, 1,000,000 ohms resistivity reconstituted to U. S. Fish and Wildlife Service Standards.
- Temperature: 55 °F
- Alkalinity: 41.04 ppm
- Calcium hardness: 17.1 ppm
- Dissolved CO₂: < 10 ppm
- PH: 7.0
- Total hardness: 51.3 ppm
- Dissolved O₂: 6.0 ppm

Purpose:
To determine the toxicity of Tordon 225 (EPA Reg. no. 464-407) to rainbow trout.
Fish Pretest History:

Upon arrival at the Laboratory, the fish were placed in a plastic enticing pool of approximately 570 gallons capacity. Water in the pool was maintained at a temperature suitable for the species of fish and aerated continuously. The water was recirculated through a sand filter approximately once per hour.

The fish were fed commercial trout chow while at the Laboratory. They were not treated with a prophylactic chemical at anytime.

No tests were made on these fish until they had undergone a minimum 10-day-observation period.

Acclimation:

Three days prior to testing, fish from 35 to 75 mm. in length were sorted from the stock tank and placed in acclimation tanks containing the quality and temperature of water to be used during the test. The fish were not fed after being taken from the stock pool.

Test Procedure:

The handling of the fish and the organization of the tests followed procedures described in Doudoroff (1951), Lenon (1964) and the Fish Pesticide Acute Toxicity Test Method as developed by the Animal Biology Staff, Pesticides Regulation Division, ARS in August 1966. Test results were analyzed and the LC 50 concentrations were computed by use of the Litchfield and Wilcoxon (1949) method.

The bio-assay tests were made in 5-gallon-glass jars containing 15 liters of reconstituted water. Fish were placed in each jar one day before the test chemicals were added. Twenty fish were tested at each concentration. The stock solutions of chemicals were mixed within 1 hour of the start of the test. The aliquot of chemical necessary to obtain the desired concentration of toxicant was added to the test jars and immediately stirred into the water to ensure an even distribution. All toxicity levels presented in this paper are based on the amount of active ingredients present in the test solutions unless indicated otherwise.

The reaction of the fish to the toxicant was recorded at elapsed times of 3/4, 1 1/2, 3, 6, 12 and 24 hours. Readings were taken at 24-hour intervals after the first day of the test period. Observations made at non-scheduled intervals were also recorded.

* Direct application of Tordon 225 to jars.

** Total formulation.
Test Results:

The analysis of the test results are presented on probit analysis sheets in the appendix. The table below summarizes some of the important information from these sheets.

The lowest limit in the 95-percent confidence interval for LC 10 and the highest limit in the 95-percent confidence interval for LC 90 at various time intervals was used to indicate the range in concentrations of a chemical that could be expected to kill fish 10-90 percent of the time.

Concentration of Tordon 225 in ppm expected by computation to kill rainbow trout.

<table>
<thead>
<tr>
<th>Test Period</th>
<th>LC&lt;sub&gt;10&lt;/sub&gt;</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>LC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr.</td>
<td>69</td>
<td>82</td>
<td>96</td>
</tr>
<tr>
<td>48 hr.</td>
<td>59</td>
<td>70</td>
<td>83</td>
</tr>
<tr>
<td>96 hr.</td>
<td>58</td>
<td>67</td>
<td>78</td>
</tr>
</tbody>
</table>

Conclusions:

Tordon 225 (EPA Reg. no. 464-407) can be expected to kill rainbow trout at a concentration of 69 ppm formulation within 24 hours of exposure.

The 24-hour LC<sub>50</sub> is 82 ppm.

Test conducted by,

John A. McCann
Biologist

Test approved by,

John A. Ludeman
Laboratory Supervisor
PESTICIDES REGULATION DIVISION
Animal Biology Section (Fish)

PROBIT ANALYSIS WORK SHEET

Analysis by: John McCann, Biologist 5/8/72

Date Tested 4/25/72

Chemical Tordon 225 MB 153

Source Wytheville NFR

Exp. Period 2 hr.

Concentration ppm. No. dead/ No. tested Observed % Mortality Expected % Mortality O-E Contributions to Chi

100 197/20 95 95 0 0
75 5/20 29 25 0 0
50 0/20 0 .06 .2 .14 0
42
32
24

Total Fish Tested = 60
Number of Doses (x) = 3
Degrees of Freedom (K-2) = 1

Total Cont. X to Chi

\[ \chi^2 = \text{Total Cont.} \times \frac{1}{K} \]

\[ \chi^2(p=.05) \text{ for } 1 \text{ deg of freedom} = 3.84 \]

1. DETERMINE \( fLC_{50} \):

\[ LCG_4 = 93 \]
\[ LCG_0 = 82 \]
\[ LCG_1 = 72 \]

\[ S = \frac{LC_{94}}{LC_{50}} + \frac{LC_{50}}{LC_{16}} = 1.14 \]

\[ N' = \frac{16\% \text{ and } 84\% \text{ E}}{2} \]

\[ fLC_{50} = S^{2.77/4.77} = S' \]

2. DETERMINE \( fS \):

\[ R (\text{Largest/Smallest dose plotted}) = 1.14 \]
\[ S (\text{As determined above}) \]
\[ A (\text{Nomo. #3 using } R \text{ and } S) \]

\[ fS = \frac{A \times (K-1)/K^{4.77}}{N'} = \frac{A}{\text{(Nomo. #2)}} \]

3. DETERMINE \( fLC_y \):

\[ (fS)^x = fS^{2.77} \text{ or 1.30 (Table 3 and Nomo. #2)} = \]

\[ fLC_y \text{ (Nomo. #4; using } (fS)^x \text{ and } fLC_{50}) = \]

4. RESULTS (\( LC_x \) and Confidence Limits at } p = .05):

\[ LC_{10} = 69 \]
\[ \text{Lower Limit } (LC_{10}/LC_y) \]
\[ \text{Upper Limit } (LC_{10} \times LC_y) \]

\[ LC_{50} = 82 \]
\[ \text{Lower Limit } (LC_{50}/fLC_{50}) \]
\[ \text{Upper Limit } (LC_{50} \times fLC_{50}) \]

\[ LC_{90} = 96 \]
\[ \text{Lower Limit } (LC_{90}/LC_y) \]
\[ \text{Upper Limit } (LC_{90} \times LC_y) \]
PESTICIDES REGULATION DIVISION
Animal Biology Section (Fish)

PROBIT ANALYSIS WORK SHEET

Chemical: Tordon 225 #MR 153

Analysis by: John McCann, Biologist 5/8/72

Exp. Period 48 hr.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>No. dead/No. tested</th>
<th>Observed % Mortality</th>
<th>Expected % Mortality</th>
<th>O-E</th>
<th>Contributions to Chi (Nomo. #1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>20/20</td>
<td>100 (99.88)</td>
<td>99.6</td>
<td>.28</td>
<td>.0022</td>
</tr>
<tr>
<td>75</td>
<td>14/20</td>
<td>70</td>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>56</td>
<td>0/20</td>
<td>0 (1.6)</td>
<td>5</td>
<td>3.4</td>
<td>.024</td>
</tr>
<tr>
<td>42</td>
<td>0/20</td>
<td>0 ( )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total Fish Tested = 60
Number of Doses (K) = 3
Degrees of freedom (K-2) = 1

Chi² = Total Cont. * Total fish = .524
Chi²(p=.05) for 1 deg of freedom = 3.84

1. DETERMINE fLC₅₀:

\[ fLC₅₀ = \frac{S^2}{\mu'} = \frac{S}{\sqrt{N'}} \]

\[ S = \frac{LC_{84}/LC₅₀ + LC₅₀/LC_{16}}{2} = 1.14 \]

\[ N'(Fish used between 16% and 84% E) = \frac{(Nomo. #2)}{= \sqrt{N'}} \]

2. DETERMINE fS:

\[ R (Largest/Smallest dose plotted) \]
\[ S (As determined above) \]
\[ A (Nomo. #3 using R and S) \]

\[ fS = A^{10(K-1)}/K\sqrt{N'} = A \quad (Nomo. #2) = \]

3. DETERMINE fLCₓ:

\[ (fS)^{x} = fS^{2} or 1.30 (Table 3 and Nomo. #2) = \]

\[ fLCₓ (Nomo. #4 using (fS)^{x} and fLC₅₀) = \]

4. RESULTS (LCₓ and Confidence Limits at p = .05):

\[ LC_{10} = 59 \]
\[ Lower Limit (LC₁₀/LCₓ) \]
\[ Upper Limit (LCₓ x LC₁₀) \]

\[ LC_{90} = 83 \]
\[ Lower Limit (LC₉₀/LCₓ) \]
\[ Upper Limit (LCₓ x LC₉₀) \]

\[ LC₅₀ = 70 \]
\[ Lower Limit (LC₅₀/fLC₅₀) \]
\[ Upper Limit (LC₅₀ x fLC₅₀) \]
PESTICIDES REGULATION DIVISION
Animal Biology Section (Fish)

PROBIT ANALYSIS WORK SHEET

Chemical Tordon 225 #MB 153

Experiment Period 96 hr.
Analysis by: John McCann, Biologist 5/8/72

<table>
<thead>
<tr>
<th>Concentration ppm</th>
<th>No. dead</th>
<th>Observed %</th>
<th>Expected %</th>
<th>O-E</th>
<th>Contributions to Chi²(Nom. #1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>20/20</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>17/20</td>
<td>85</td>
<td>85</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>56</td>
<td>0/20</td>
<td>0 (1.6)</td>
<td>5</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>0/20</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Fish Tested =</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of Doses (K) = Total fish = Total fish =

Degrees of freedom (K-2) = K

Chi² = Total Cont. x to Chi² = N' (Fish used between 16% and 84% E) =

Chi²(p=.05) for deg of freedom =

1. DETERMINE fLC₅₀:

\[
\begin{align*}
\text{LC}_{16} & = 75 \\
\text{LC}_{50} & = 67 \\
\text{LC}_{75} & = 60 \\
S & = \frac{\text{LC}_{04} / \text{LC}_{75} + \text{LC}_{75} / \text{LC}_{16}}{2} \\
N' & = \sqrt{N'} (\text{Fish used between 16% and 84% E}) = \\
\text{fLC}_{50} & = \frac{S^2}{\sqrt{N'}} = S. \\
\end{align*}
\]

(Nom. #2) =

2. DETERMINE fS:

\[
\begin{align*}
R & \quad \text{(Largest/Smallest dose plotted)} \\
S & \quad \text{(As determined above)} \\
A & \quad \text{(Nom. #3 using R and S)} \\
fS & = A^{(K-1) / K \sqrt{N'}} = A. \\
\end{align*}
\]

(Nom. #2) =

3. DETERMINE fLCₙ:

\[
\begin{align*}
(fS)^x & = fS^{2.33} \text{ or } 1.30 (\text{Table 3 and Nom. #2}) = \\
fLC_u (\text{Nom. #4 using } (fS)^x \text{ and fLC}_{50}) = \\
\end{align*}
\]

4. RESULTS (LCₙ and Confidence Limits at p = .05):

\[
\begin{align*}
\text{LC}_{16} & = 58 \\
\text{LC}_{50} & = 67 \\
\text{LC}_{75} & = 78 \\
\end{align*}
\]

(Lower Limit) LCₙ / (Upper Limit) LCₙ =

(Lower Limit) LCₙ X (Upper Limit) LCₙ =
LITERATURE CITED


