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FISH TOXICITY LABORATORY REPORT

Animal Biology Laboratory
ARS-PR, ARC, Beltsville, Md.

Test Number: 477

I.D. Number: MB 153
USDA Reg. No.: 464-407

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%
Inert ingredients:	69.9%

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.
Water volume: 15 l.
Fish/vessel: 10
Fish/concentration: 20
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	**pH:**	7.0
Alkalinity:	41.04 ppm.	**Total hardness:**	51.3 ppm.
Calcium hardness:	17.1 ppm.	**Dissolved O_2:**	6.0 ppm.
Dissolved CO_2:	< 10 ppm.		

Purpose:

To determine the toxicity of Tordon 225 (EPA Reg. no. 464-407) to rainbow trout.

1/81

Fish Pretest History:

Upon arrival at the Laboratory, the fish were placed in a plastic swimming 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), Lennon (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 the active ingredient that could be expected to kill from 10-90 percent of the fish 95 percent of the time.

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

Test Period	LC_{10}	LC_{50}	LC_{90}
24 hr.	69	82	96
48 hr.	59	70	83
96 hr.	58	67	78

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_{50} 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

Test Species Rainbow trout
Source Wytheville NFH
Exp. Period 2- hr.
Chemical Tordon 225 MB 153
Date Tested 4/25/72

Analysis by: John McCann, Biologist 5/8/72
(Name) (Title) (Date)

Concentration ppm.	No. dead/ /No. tested	Observed % Mortality	Expected % Mortality	O-E	Contributions to Chi (Nomo #1)
100	19/20	95	95	0	0
75	5/20	25	25	0	0
56	0/20	0 (.06)	.2	.14	0
42					
32					
24					

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

Chi^2 = Total Cont. to Chi X $\frac{\text{Total fish}}{K}$ = 00

Chi^2(p=.05) for 1 deg of freedom = 3.84

1. DETERMINE fLC_{50}:

LC_{84} 93
LC_{50} 82
LC_{16} 72

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$ = 1.14

$fLC_{50} = S^{2.77/\sqrt{N'}}$ = S-_____

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

2. DETERMINE fS:

R (Largest/Smallest dose plotted) _____
S (As determined above) 1.14
A (Nomo. #3 using R and S) _____

$fS = A^{10(K-1)/K\sqrt{N'}}$ = A_____ (Nomo. #2) = _____

3. DETERMINE fLC_y:

$(fS)^X = fS^{2.65}$ or 1.30 (Table 3 and Nomo. #2) = _____

fLC_y (Nomo. #4 using $(fS)^X$ and fLC_{50}) = _____

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

LC_{10} = 69
Lower Limit (LC_1/LC_y) _____
Upper Limit (LC_1 X LC_y) _____

LC_{50} = 82
Lower Limit (LC_{50}/fLC_{50}) _____
Upper Limit (LC_{50} X fLC_{50}) _____

LC_{90} = 96
Lower Limit (LC_{90}/LC_y) _____
Upper Limit (LC_{90} X LC_y) _____

PESTICIDES REGULATION DIVISION
Animal Biology Section (Fish)
PROBIT ANALYSIS WORK SHEET

Test Species Rainbow trout
Source Wytheville NFH
Exp. Period 48 hr.

Chemical Tordon 225 #MB 153
Date Tested 4/25/72

Analysis by: John McCann, Biologist 5/8/72
(Name) (Title) (Date)

Concentration ppm	No. dead/ /No. tested	Observed % Mortality	Expected % Mortality	O-E	Contributions to Chi(Nomo #1)
100	20/20	100 (99.88)	99.6	.28	.0022
75	14/20	70	70	0	00
56	0/20	0 (1.6)	5	3.4	.024
42	0/20	0 ( )			
32					
24					

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

Total .0262
Chi^2 = Total Cont. to Chi X Total fish / K = .524
Chi^2 (p=.05) for 1 deg of freedom = 3.84

1. DETERMINE fLC_{50}:

LC_{84} 80
LC_{50} 70
LC_{16} 61

$S = LC_{84}/LC_{50} + LC_{50}/LC_{16} / 2$ = 1.14

N'(Fish used between 16% and 84% E) =

$fLC_{50} = S^{2.77/\sqrt{N'}}$ = S_____ $\sqrt{N'}$ = (Nomo. #2) =

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_____ (Nomo. #2) =

3. DETERMINE fLC_y:

$(fS)^X = fS^{2}$ or 1.30 (Table 3 and Nomo. #2) =

fLC_y (Nomo. #4 using $(fS)^X$ and fLC_{50}) =

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

LC_{10} = 59
Lower Limit (LC_{10}/LC_y)
Upper Limit (LC_{10} X LC_y)

LC_{50} = 70
Lower Limit (LC_{50}/fLC_{50})
Upper Limit (LC_{50} X fLC_{50})

LC_{99} = 83
Lower Limit (LC_{99}/LC_y)
Upper Limit (LC_{99} X LC_y)

PESTICIDES REGULATION DIVISION
Animal Biology Section (Fish)
PROBIT ANALYSIS WORK SHEET

Test Species Rainbow trout
Source Wytheville NFH
Exp. Period 96 hr.

Chemical Tordon 225 #MB 153
Date Tested 4/25/72

Analysis by: John McCann, Biologist 5/8/72
(Name) (Title) (Date)

Concentration ppm	No. dead/ /No. tested	Observed % Mortality	Expected % Mortality	O-E	Contributions to Chi (Nomo #1)
100	20/20	100 ( )			
75	17/20	85	85	0	0
56	0/20	0 (1.6)	5	3.4	
42	0/20	0			
32					
24					

Total Fish Tested = _____
Number of Doses (K) = _____
Degrees of freedom (K-2) = _____

Chi^2 = Total Cont. to Chi X $\frac{\text{Total Total fish}}{K}$ = _____

Chi^2 (p=.05) for ____ deg of freedom = _____

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1. DETERMINE fLC_{50}:

LC_{84} 75
LC_{50} 67
LC_{16} 60

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$ = _____

N'(Fish used between 16% and 84% E) = _____

$\sqrt{N'}$ = _____

$fLC_{50} = S^{2.77/\sqrt{N'}} = S$-_____ (Nomo. #2) = _____

2. DETERMINE fS:

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

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

3. DETERMINE fLC_y:

$(fS)^X = fS^{2.33}$ or 1.30 (Table 3 and Nomo. #2) = _____

fLC_y (Nomo. #4 using $(fS)^X$ and fLC_{50}) = _____

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

LC_{10} = 58
Lower Limit (LC_{10}/LC_y) _____
Upper Limit (LC_{10} X LC_y) _____

LC_{50} = 67
Lower Limit (LC_{50}/fLC_{50}) _____
Upper Limit (LC_{50} X fLC_{50}) _____

LC_{90} = 78
Lower Limit (LC_{90}/LC_y) _____
Upper Limit (LC_{90} X LC_y) _____

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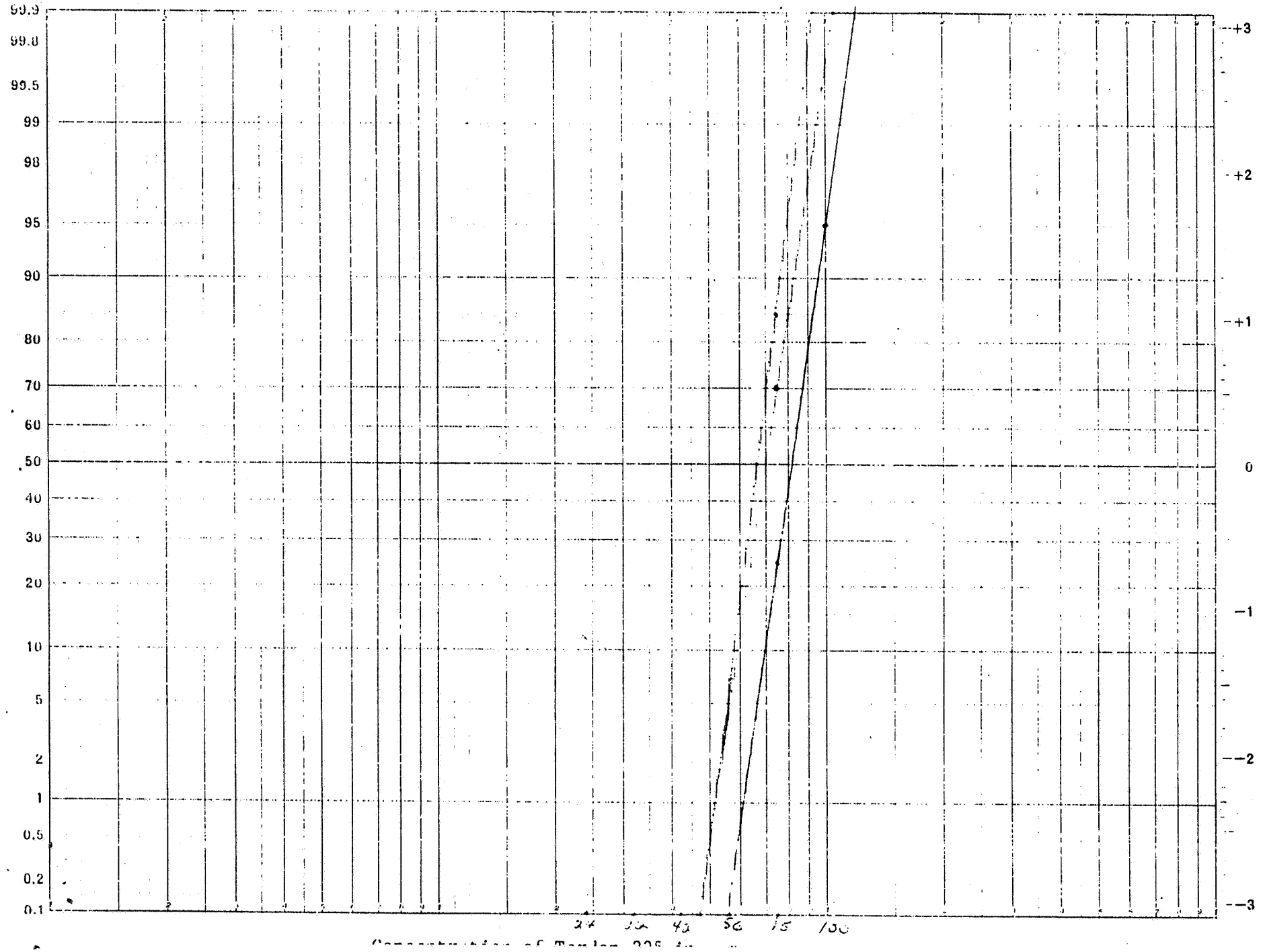
GRAPH PAPER

Toxicity of Tordon 225 to rainbow trout.



Concentration of Tordon 225 in