

Rotenone Reregistration  
EPA Administrative Number 6704-Q  
Wildlife and Aquatic Organisms Data  
January 23, 1987

VOLUME 2 ROTENONE--FRESHWATER FISH LC50--  
RAINBOW TROUT AND BLUEGILLS

(Guideline No. 72-1[a] and [b])

Study Title

Rotenone--Freshwater Fish LC50--Rainbow Trout and Bluegills

Data Requirement

Guideline No. 72-1(a) and (b)

Authors

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Study Completed On

August 1986

Performing Laboratory

U.S. Fish and Wildlife Service  
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Laboratory Project ID

TOX 83-626.01B

Supplemental Submissions

Resubmission of

Study Title: Fish Acute LC50--Rainbow Trout and Bluegill  
(40 CFR 163.72-1)  
Fish Embryolarval Study--Rainbow Trout (40 CFR 163.72-4)

Data Requirement: Guideline No. 72-1(a) and (b)

Authors: Not listed, but were L. L. Marking and T. D. Bills

Study Date: November 5, 1982

Accession Number: 248788

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Agency U.S. Fish and Wildlife Service

Agency Agent: Fred P. Meyer Date: 01/23/87

Quality Assurance Officer   
Signature

GOOD LABORATORY PRACTICE STATEMENT

This study meets the requirements for 40 CFR Part 160

Submitter National Fisheries Research Center-La Crosse

Sponsor U.S. Fish and Wildlife Service

Study Director Leif L. Marking

## SUMMARY

Rotenone, a crystalline ketone found in several plants of the family Leguminosae, has been used extensively as an insecticide and a piscicide. The chemical has been used by fishery managers since the 1930's for the removal of undesirable fish populations from lakes and streams. The use of rotenone as a fishery management tool stems from its high toxicity to fish, low toxicity to mammals, and rapid decomposition in the environment (Lennon et al. 1970; Kaley 1978). The toxicity of rotenone to aquatic organisms has been studied extensively (Schnick 1974; Marking and Bills 1976). However, recent changes in the U.S. Environmental Protection Agency (EPA) regulations regarding toxic effects on aquatic organisms exposed to chemicals and a reevaluation of existing data files for certain chemicals prompted the Fish and Wildlife Service (FWS) to undertake several additional studies to demonstrate safe use of rotenone as a piscicide.

The present study was designed to determine the acute toxicity to rainbow trout (Salmo gairdneri) and bluegills (Lepomis macrochirus) under standard test conditions.

Rotenone formulations used for this study were supplied by S. B. Penick and Company; technical rotenone was crystalline and had a purity of 96.47%, Noxfish<sup>®1</sup> and Nusyn-Noxfish were liquids and contained 5% and 2.5% rotenone, respectively. Crystalline rotenone was solubilized in acetone and Noxfish and Nusyn-Noxfish were solubilized in water.

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<sup>1</sup>Use of trade names does not imply U.S. Government endorsement of commercial products.

Rainbow trout for this study were obtained as eggs from the Ennis National Fish Hatchery and cultured at the National Fisheries Research Center (NFRC), La Crosse, Wisconsin. Bluegills for the study were cultured at the NFRC. Rainbow trout were 34.7 mm in length and weighed 0.45 g; bluegills were 33.1 mm in length and weighed 0.59 g. Fish were handled according to procedures outlined by Hunn et al. (1968). Observations on survival and mortality were conducted at 1, 3, and 6 hours on the first day and daily thereafter, and dead organisms were removed.

Tests were done in accordance with methods outlined by ASTM Committee E-35 on Pesticides (1980) and Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Exposures were replicated and a total of 20 organisms was exposed to each concentration. Tests were conducted at 12°C in soft water (total hardness, 44; range, 40-48 expressed as mg/L CaCO<sub>3</sub>; alkalinity, 32; range 30-35 expressed as mg/L CaCO<sub>3</sub>; conductivity, 163; range 160-165; pH, 7.5; range 7.2-7.6; dissolved oxygen, 10.8 mg/L; lighting 12/12). Mortality data were analyzed for LC<sub>50</sub> values (concentrations calculated to produce 50% mortality), and 95% confidence intervals according to the method of Litchfield and Wilcoxon (1949).

The 96-hour LC<sub>50</sub>'s for rainbow trout ranged from 2.82 µg/L for the technical rotenone to 49.0 µg/L for Noxfish (Table 1). With bluegills, the 96-hour LC<sub>50</sub>'s ranged from 6.09 µg/L for the technical rotenone to 153 µg/L for Noxfish (Table 2). Comparison of the activity of the three formulations corrected to 100% active ingredient showed the Nusyn-Noxfish formulation to be approximately twice as toxic to both species as were technical rotenone and Noxfish (Table 3). No significant differences were found between any of the replicate tests.

TECHNICAL SUMMARY PAGE

Compound: Rotenone

Formulation: Technical, 96.47%; A. I., crystalline  
Noxfish, 5.0%; A. I., liquid  
Nusyn-Noxfish, 2.5%; A. I., Liquid

Batch No.: Technical, 215-LE0-1  
Noxfish, 1125-LEA-2  
Nusyn-Noxfish, 6425-LCA-5

Solvent: Technical, Acetone  
Noxfish, Water  
Nusyn-Noxfish, Water

Source: S.B. Penick and Company

Study: Acute toxicity of three rotenone formulations to rainbow trout (Salmo gairdneri) and bluegills (Lepomis macrochirus)

Results: Rainbow trout and bluegills were exposed to technical rotenone, Noxfish, and Nusyn-Noxfish in static tests for 96 hours in soft water at 12°C. Tests were conducted in replicate and data analyzed for LC50's (concentrations calculated to produce 50% mortality) and 95% confidence intervals. The 96-hour LC50's for rainbow trout were: technical rotenone--Group A, 2.83 µg/L (2.28-3.51); Group B, 2.82 µg/L (2.27-3.49); Nusyn-Noxfish--Group A, 36.2 µg/L (25.2-52.0); Group B, 48.9 µg/L (43.1-55.4); Noxfish--Group A, 49.0 µg/L (43.2-55.6); Group B, 45.0 µg/L (35.5-57.0). The 96-hour LC50's for bluegills were: technical rotenone--Group A, 6.32 µg/L (5.14-7.78); Group B, 6.09 µg/L (5.39-6.88); Nusyn-Noxfish--Group A, 142 µg/L (115-176); Group B, 122 µg/L (94.7-157); Noxfish--Group A, 153 µg/L (115-203); Group B, 117 µg/L (86.9-157). There were no significant differences between any replicate tests. Comparison of the activity of the three formulations corrected to 100% active ingredient showed the Nusyn-Noxfish formulation to be approximately twice as toxic to both species as were technical rotenone and Noxfish. The corrected 96-hour LC50's for rainbow trout were: technical rotenone--Group A, 2.73 µg/L (2.20-3.39); Group B, 2.72 µg/L (2.19-3.37); Nusyn-Noxfish--Group A, 0.905 µg/L (0.630-1.30); Group B, 1.22 µg/L (1.08-1.38); Noxfish--

Group A, 2.45 µg/L (2.16-2.78); Group B, 2.25 µg/L (1.77-2.85). The corrected 96-hour LC50's for bluegills were: technical rotenone--Group A, 6.09 µg/L (4.96-7.50); Group B, 5.87 µg/L (5.20-6.64); Nusyn-Noxfish--Group A, 3.55 µg/L (2.87-4.40); Group B, 3.05 µg/L (2.36-3.92); Noxfish--Group A, 7.60 µg/L (5.75-10.1); Group B, 5.85 µg/L (4.34-7.85).

References: ASTM Committee E-35 on Pesticides. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729080. In Annual Book of ASTM Standards, Part 46, pp. 1-25. End use and consumer products, American Society for Testing and Materials, Philadelphia, Pennsylvania.

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Table 1. Toxicity of three formulations of rotenone to rainbow trout (*Salmo gairdneri*) in soft water at 12°C.

Formulation and % active ingredient	LC50 (µg/L) and 95% confidence interval at											
	1 hour		3 hours		6 hours		24 hours		95 hours			
	A*	B	A	B	A	B	A	B	A	B		
Technical rotenone (96.47%)	8.45 7.66-9.32	8.99 7.10-11.4	6.55 5.80-7.40	6.58 5.84-7.41	6.39 5.63-7.25	6.00 5.36-6.71	3.79 3.15-4.56	3.85 3.02-4.91	2.83 2.28-3.51	2.82 2.27-3.49		
Musyn-Noxfish (2.5%)	362 252-520	340 232-498	210 170-259	142 114-176	163 133-200	106 88.5-127	62.0 54.5-70.5	69.5 63.6-76.0	36.2 25.2-52.0	48.9 43.1-55.4		
Noxfish (5.0%)	246 190-318	233 183-296	140 113-173	141 113-175	112 94.7-132	99.0 81.1-121	54.0 48.0-60.7	55.0 49.6-61.0	49.0 43.2-55.6	45.0 35.5-57.0		

\*Replicate tests.

Table 2. Toxicity of three formulations of rotenone to bluegills (Lepomis macrochirus) in soft water at 12°C.

Formulation and % active ingredient		LC50 (µg/L) and 95% confidence interval at											
		1 hour		3 hours		6 hours		24 hours		96 hours			
A*	B	A	B	A	B	A	B	A	B	A	B		
Technical rotenone (96.47%)	>40.0	24.4 21.5-27.7	36.6 31.0-38.6	24.2 21.3-27.5	24.6 21.7-27.9	9.20 6.95-12.2	9.00 7.07-11.5	6.32 5.14-7.78	6.09 5.39-6.88				
Nusyn-Noxfish (2.5%)	>600	>600	>600	645 562-740	>600	246 195-311	258 215-309	142 115-176	122 94.7-157				
Noxfish (5.0%)	>600	>600	510 449-579	>600	429 389-473	200 141-283	155 113-211	153 115-203	117 86.9-157				

\*Replicate tests.

Table 3. Comparison of the toxicity of three formulations of rotenone corrected to 100% active ingredient to rainbow trout (Salmo gairdneri) and bluegills (Lepomis macrochirus) in soft water at 12°C.

Formulation	% A. I.	Rainbow trout		Bluegills	
		A*	B	A	B
Technical rotenone	96.47	2.73 2.20-3.39	2.72 2.19-3.37	6.09 4.96-7.50	5.87 5.20-6.64
Nusyn-Noxfish	2.5	0.905 0.630-1.30	1.22 1.08-1.38	3.55 2.87-4.40	3.05 2.36-3.92
Noxfish	5.0	2.45 2.16-2.78	2.25 1.77-2.85	7.60 5.75-10.1	5.85 4.34-7.85

\*Replicate tests.

Appendix I

Table 1. Observed mortality among 10 rainbow trout (*Salmo gairdneri*) exposed to technical rotenone (96.47%) in soft water at 12°C.

Concentration (µg/L)	% mortality at													
	1 hour		3 hours		6 hours		24 hours		48 hours		72 hours		96 hours	
	A	B <sup>a</sup>	A	B	A	B	A	B	A	B	A	B	A	B
Control (0)	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
Acetone control (0)	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
0.5	0/0		0/0		0/0		0/0		0/0		0/10		0/10	
1	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
2	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
4	0/0		0/0		0/0		60/100		80/100		100/100		100/100	
6	0/0		10/10		20/50		100/100		80/100		100/100		100/100	
8	10/0		100/100		100/100		100/100		100/100		100/100		100/100	
10	100/100		100/100		100/100		100/100		100/100		100/100		100/100	
20	100/100		100/100		100/100		100/100		100/100		100/100		100/100	

<sup>a</sup>Replicate tests.

96-hour LC50: A = 2.83 µg/L (2.28-3.51); B = 2.82 µg/L (2.27-3.49).

Observed no effect level: A = 2 µg/L; B = 2 µg/L.

Table 2. Observed mortality among 10 rainbow trout (*Salmo gairdneri*) exposed to Nusyn-Noxfish (2.5% rotenone) in soft water at 12°C.

Concentration (µg/L)	1 hour		3 hours		6 hours		24 hours		48 hours		72 hours		96 hours	
	A	B <sup>a</sup>	A	B	A	B	A	B	A	B	A	B	A	B
Control (0)	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
5	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
10	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
20	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
40	0/0		0/0		0/0		0/0		10/0		20/0		20/0	
60	0/0		0/0		0/0		30/10		100/100		100/100		100/100	
80	0/0		0/0		0/0		30/90		10/100		100/100		100/100	
100	0/0		0/0		0/40		100/100		100/100		100/100		100/100	
200	0/0		40/100		90/100		100/100		100/100		100/100		100/100	
400	70/80		100/100		100/100		100/100		100/100		100/100		100/100	

<sup>a</sup>Replicate tests.

96-hour LC50: A = 36.2 µg/L (25.2-52.0); B = 48.9 µg/L (43.1-55.4).

Observed no effect level: A = 20 µg/L; B = 40 µg/L.

Table 3. Observed mortality among 10 rainbow trout (*Salmo gairdneri*) exposed to Noxfish (5% rotenone) in soft water at 12°C.

Concentration (µg/L)	% mortality at						
	1 hour A B <sup>a</sup>	3 hours A B	6 hours A B	24 hours A B	48 hours A B	72 hours A B	96 hours A B
Control (0)	0/0	0/0	0/0	0/0	0/10	0/10	0/10
5	0/0	0/0	0/0	0/0	0/0	0/0	0/0
10	0/0	0/0	0/0	0/0	0/0	0/0	0/0
20	0/0	0/0	0/0	0/0	0/0	0/0	0/0
40	0/0	0/0	0/0	0/0	10/0	10/0	10/0
60	0/0	0/0	0/0	80/90	100/100	100/100	100/100
80	0/0	0/0	0/0	100/100	100/100	100/100	100/100
100	0/0	0/0	60/30	100/100	100/100	100/100	100/100
200	20/10	100/100	100/100	100/100	100/100	100/100	100/100
400	100/100	100/100	100/100	100/100	100/100	100/100	100/100

<sup>a</sup>Replicate tests.

96-hour LC50: A = 49.0 µg/L (43.2-55.6); B = 45.0 µg/L (35.5-57.0).

Observed no effect level: A = 20 µg/L; B = 40 µg/L.

Table 4. Observed mortality among 10 bluegills (*Lepomis macrochirus*) exposed to technical rotenone (96.47%) in soft water at 12°C.

Concentration (µg/L)	1 hour		3 hours		6 hours		24 hours		48 hours		72 hours		96 hours	
	A	B <sup>a</sup>	A	B	A	B	A	B	A	B	A	B	A	B
Control (0)	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
Acetone control (0)	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
2.5	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
5.0	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
7.5	0/0		0/0		10/0		30/20		30/40		30/70		40/100	
10	0/0		0/0		0/0		60/70		70/80		90/100		90/100	
15	0/0		0/0		0/0		60/80		80/80		90/100		100/100	
20	0/0		10/0		10/0		100/100		100/100		100/100		100/100	
30	0/0		90/0		100/100		100/100		100/100		100/100		100/100	
40	0/0		100/100		100/100		100/100		100/100		100/100		100/100	

<sup>a</sup>Replicate tests.

96-hour LC50: A = 6.32 µg/L (5.14-7.78); B = 6.09 µg/L (5.39-6.88).

Observed no effect level: A = 5 µg/L; B = 5 µg/L.



Table 5. Observed mortality among 10 bluegills (*Lepomis macrochirus*) exposed to Nusyn-Noxfish (2.5% rotenone) in soft water at 12°C.

Concentration (µg/L)	% mortality at						
	1 hour A B <sup>a</sup>	3 hours A B	6 hours A B	24 hours A B	48 hours A B	72 hours A B	96 hours A B
Control (0)	0/0	0/0	0/0	0/0	0/0	0/0	0/0
25	10/0	10/0	10/0	10/0	10/0	10/0	10/0
50	0/0	0/0	0/0	0/0	0/0	0/0	0/0
75	0/0	0/0	0/0	0/0	0/0	0/0	0/0
100	0/0	0/0	0/0	0/0	0/0	0/10	0/10
200	0/0	0/0	0/0	30/10	80/90	80/100	100/100
300	0/0	0/0	0/0	70/80	100/100	100/100	100/100
400	0/0	0/0	0/0	100/90	100/100	100/100	100/100
500	0/0	0/0	0/0	100/100	100/100	100/100	100/100
600	0/0	0/0	30/0	100/100	100/100	100/100	100/100

<sup>a</sup>Replicate tests.

96-hour LC50: A = 142 µg/L (115-176); B = 122 µg/L (94.7-157).

Observed no effect level: A = 75 µg/L; B = 75 µg/L.

Table 6. Observed mortality among 10 bluegills (Lepomis macrochirus) exposed to Noxfish (5% rotenone) in soft water at 12°C.

Concentration (µg/L)	% mortality at													
	1 hour		3 hours		6 hours		24 hours		48 hours		72 hours		96 hours	
	A	B <sup>a</sup>	A	B	A	B	A	B	A	B	A	B	A	B
Control (0)	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
25	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
50	0/0		0/0		0/0		0/0		0/0		0/0		0/0	
75	0/0		0/0		0/0		0/10		0/10		0/10		0/10	
100	0/0		0/0		0/0		10/20		10/20		10/20		10/20	
200	0/0		0/0		0/0		50/30		80/90		80/90		80/100	
300	0/0		0/0		0/0		90/100		100/100		100/100		100/100	
400	0/0		0/10		0/10		100/100		100/100		100/100		100/100	
500	0/0		0/20		0/100		100/100		100/100		100/100		100/100	
600	0/0		0/100		0/100		100/100		100/100		100/100		100/100	

<sup>a</sup>Replicate tests.

96-hour LC50: A = 153 µg/L (115-203); B = 117 µg/L (86.9-157).

Observed no effect level: A = 75 µg/L; B = 50 µg/L.