

John

# INVESTIGATIONS IN FISH CONTROL

## 72. Toxicity of Rotenone to Fish in Standardized Laboratory Tests



United States Department of the Interior  
Fish and Wildlife Service

Investigations in Fish Control, published by the Fish and Wildlife Service, include reports on the results of work at the Service's Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., and reports of other studies related to that work. Though each report is regarded as a separate publication, several may be issued under a single cover, for economy. [See Investigations in Fish Control 47-50 (in one cover) for list of issues published prior to 1970.]

(Reports 39 and 40 are in one cover.)

39. Effects of Antimycin A on Tissue Respiration of Rainbow Trout and Channel Catfish, by Richard A. Schoettger and Gerald E. Svendsen. 1970. 10 pp.
40. A Resume on Field Applications of Antimycin A to Control Fish, by Robert E. Lennon and Bernard L. Berger. 1970. 19 pp.

(Reports 41 through 43 are in one cover.)

41. Identification of MS-222 Residues in Selected Fish Tissues by Thin Layer Chromatography, by John L. Allen, Charles W. Luhning, and Paul D. Harman. 1970. 7 pp.
42. Dynamics of MS-222 in the Blood and Brain of Freshwater Fishes During Anesthesia, by Joseph B. Hunn. 1970. 8 pp.
43. Effect of MS-222 on Electrolyte and Water Content in the Brain of Rainbow Trout, by Wayne A. Willford. 1970. 7 pp.
44. A Review of Literature on TFM (3-trifluormethyl-4-nitrophenol) as a Lamprey Larvicide, by Rosalie A. Schnick. 1972. 31 pp.

(Reports 45 and 46 are in one cover.)

45. Residues of MS-222 in Northern Pike, Muskellunge, and Walleye, by John L. Allen, Charles W. Luhning, and Paul D. Harman. 1972. 8 pp.
46. Methods of Estimating the Half-Life of Biological Activity of Toxic Chemicals in Water, by Leif L. Marking. 1972. 9 pp.

(Reports 47 through 50 are in one cover.)

47. Preparation and Properties of Quinaldine Sulfate, an Improved Fish Anesthetic, by John L. Allen and Joe B. Sills. 1973. 7 pp.
48. Toxicity of Quinaldine Sulfate to Fish, by Leif L. Marking and Verdel K. Dawson. 1973. 8 pp.
49. The Efficacy of Quinaldine Sulfate as an Anesthetic for Freshwater Fish, by Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman. 1973. 9 pp.
50. Residue of Quinaldine in Ten Species of Fish Following Anesthesia with Quinaldine Sulfate, by Joe B. Sills, John L. Allen, Paul D. Harman, and Charles W. Luhning. 1973. 9 pp.

(Reports 51 and 52 are in one cover.)

51. Methods for Simultaneous Determination and Identification of MS-222 and Metabolites in Fish Tissues, by Charles W. Luhning. 1973. 10 pp.
52. Residues of MS-222, Benzocaine, and Their Metabolites in Striped Bass Following Anesthesia, by Charles W. Luhning. 1973. 11 pp.

(Reports 53 through 55 are in one cover.)

53. Toxicity of Mixtures of Quinaldine Sulfate and MS-222 to Fish, by Verdel K. Dawson and Leif L. Marking. 1973. 11 pp.
54. The Efficacy of Quinaldine Sulfate:MS-222 Mixtures for the Anesthetization of Freshwater Fish, by Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman. 1973. 9 pp.
55. Residues of Quinaldine and MS-222 in Fish Following Anesthesia with Mixtures of Quinaldine Sulfate:MS-222, by Joe B. Sills, John L. Allen, Paul D. Harman, and Charles W. Luhning. 1973. 12 pp.

# INVESTIGATIONS IN FISH CONTROL

## 72. Toxicity of Rotenone to Fish in Standardized Laboratory Tests

By **Leif L. Marking**

**Terry D. Bills**



**United States Department of the Interior**

**Fish and Wildlife Service**

**Washington, D.C. • 1976**

## Contents

|  | <b>Page</b> |
|--|-------------|
| Abstract .....   | 1           |
| Materials and Methods.....                                       | 1           |
| Results .....  | 2           |
| Toxicity to various species of fish .....                        | 2           |
| Effects of temperature, water hardness, and pH on toxicity ..... | 2           |
| Toxicity of Noxfish to green eggs of rainbow trout.....          | 2           |
| Persistence of Noxfish in water .....                            | 6           |
| Detoxification of Noxfish .....                                  | 8           |
| Toxicity of different formulations of rotenone.....              | 8           |
| Toxicity in flow-through tests .....                             | 8           |
| Discussion .....   | 10          |
| References .....   | 11          |

# Toxicity of Rotenone to Fish in Standardized Laboratory Tests

by

Leif L. Marking and Terry D. Bills

Fish Control Laboratory, P.O. Box 862  
La Crosse, Wisconsin 54601

## Abstract

Noxfish®, which contains 5% rotenone, was toxic to a variety of freshwater fish at concentrations ranging from 21.5 to 497  $\mu$ g/l in 96-h laboratory exposures. Goldfish (*Carassius auratus*) and black bullheads (*Ictalurus melas*) were the most resistant species, and the Atlantic salmon (*Salmo salar*) was the most sensitive. Toxicity was influenced little by temperatures of 7 to 22 C, by water hardnesses of 10 to 300 mg/l, or by pH's of 6.5 to 9.5. In exposures of rainbow trout (*Salmo gairdneri*), newly fertilized eggs were much more resistant than fingerlings. Noxfish detoxified in water solutions; the half-life of biological activity was 22 days at 12 C and 13 days at 17 C. Potassium permanganate was an excellent detoxifier; chlorine was less efficient. Noxfish was consistently more toxic in static tests than in flow-through tests.

Rotenone, a crystalline ketone found in several plants of the Leguminosae, has been used as a toxicant by fishery managers since the 1930's for removing undesired fish populations in lakes (Baker and Cordone 1969). Ideal conditions for the reclamation of static waters with rotenone include temperatures between 16 and 21 C, alkalinities between 150 and 200 mg/l (ppm), pH's of 8 or less, and surface areas of less than 8.1 ha (Spitler 1970). The piscicide has been used extensively under a wide variety of conditions and is relatively harmless to most nontarget organisms (Schnick 1974; Lennon et al. 1970). Twenty-nine formulations of rotenone from 18 companies had been registered for aquatic or agricultural use by 1974 by the Environmental Protection Agency. Because the registrations are old, data supporting the labels must be updated to conform to present requirements (Lennon 1967). A guide or protocol for evaluating the toxicity of candidate fishery chemicals for registration was published by Marking (1975).

The present study was designed to determine (1) the toxicity of rotenone to fish in standardized static and flow-through tests, (2) the toxicity of rotenone to newly fertilized trout eggs, (3) the residual toxicity of rotenone in water after selected periods of aging, (4) the efficiency of two compounds used to detoxify rotenone, and (5) the comparative toxicities of three rotenone formulations.

## Materials and Methods

Three formulations of rotenone (furnished by S. B. Penick & Co.) were used in the tests described here: Noxfish®, an emulsifiable concentrate containing 5% rotenone; Pro-Noxfish®, a synergized emulsifiable concentrate containing 2.5% rotenone; and rotenone, a powder containing 33% rotenone. Except for tests in which the toxicities of the different formulations were compared or evaluated, Noxfish was used in all tests, and concentrations were based on the total amount of Noxfish formulation rather than on the amount of rotenone in the formulation. Stock solutions of the toxicants were prepared daily, and the portions needed to yield the desired concentrations were added to test chambers.

Static and flow-through test procedures followed those of Lennon and Walker (1964) and The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). In static tests, 10 fish were exposed to each concentration in glass jars containing 15 liters of oxygen-saturated test water prepared from deionized water (Marking 1969). Waters of four levels of hardness were used (total hardness as mg/l of  $\text{CaCO}_3$  in parentheses): very soft (10), soft (44), hard (170), and very hard (300).

In separate tests to assess the effect of pH on toxicity, chemical buffers were added to control the pH (Marking 1975). Test temperatures were regulated

by immersing the test jars in constant temperature water baths. In flow-through toxicity tests, 20 fish were exposed to each concentration in a system similar to that described by Mount and Brungs (1967). Modifications included electronic microswitches to control cycling, pressure regulators, and an automatic pipette (Micromedic®) for injecting the toxicants into dilution water. Municipal water used for flow-through exposures was carbon filtered and had a total hardness of 320 mg/l and a pH of 7.5. Flow rates were maintained at about five chamber volumes per 24 h. Temperature was maintained with a water bath around the test chambers.

Fish weighing 1 to 1.5 g each were obtained from Federal hatcheries and maintained according to standardized procedures of the Fish Control Laboratory (Hunn et al. 1968). Scientific names for all species used are listed in Table 1. Fish were acclimated to the test conditions for 4 days before they were exposed to the toxicant. Mortalities were recorded at 1, 3, and 6 h on the first day of exposure and daily thereafter for the remainder of the test. Trout eggs were exposed to Noxfish in a manner similar to that used for fish, except that the static test vessel contained 2.5 liters of test solution. Details of methods for exposing eggs were those outlined by Olson and Marking (1973).

The methods of Litchfield and Wilcoxon (1949) were used in computation of the  $LC_{50}$ 's (concentrations producing 50% mortality) and 95% confidence intervals. Regressions were drawn and inspected for each set of data. All data reported fulfilled the chi-square test requirement for acceptability.

Deactivation indices for Noxfish were derived in soft water at temperatures of 12 and 17 C. Aged solutions of the toxicant were bioassayed to determine the biological activity remaining after selected time periods. The deactivation index was determined by dividing the  $LC_{50}$  of aged solutions by the  $LC_{50}$  of unaged solutions under corresponding test conditions (Marking 1972). The deactivation index was plotted against aging time on semilogarithmic coordinates to estimate the half-life of biological activity. Detoxification procedures with potassium permanganate ( $KMnO_4$ ) and chlorine were those used by Marking and Bills (1975).

## Results

### *Toxicity to Various Species of Fish*

Noxfish was toxic to a wide variety of fish at concentrations ranging, in 96-h exposures, from 21.5  $\mu$ g/l (or parts per billion) for Atlantic salmon to

497  $\mu$ g/l for goldfish (Table 1). The 96-h  $LC_{50}$  was less than 100  $\mu$ g/l for bowfins, all six salmonids, northern pike, carp, longnose and white suckers, smallmouth bass, yellow perch, and walleyes. The 96-h  $LC_{50}$  was greater than 100  $\mu$ g/l for goldfish, fathead minnows, black bullheads, channel catfish, green sunfish, bluegills, and largemouth bass. Goldfish and black bullheads were most resistant—10 times as resistant as most other species. Generally, the resistant species required longer exposures than did the sensitive species, before they succumbed. None of the goldfish died in 24 h of exposure to high concentrations of Noxfish and none of the black bullheads in 6 h. On the other hand, most of the sensitive species died in 3-h exposures to much lower concentrations.

### *Effects of Temperature, Water Hardness, and pH on Toxicity*

Noxfish was generally less toxic to rainbow trout, channel catfish, and bluegills at the lower than at the higher temperatures in 3- and 6-h exposures (Tables 2, 3, and 4). After 96 h this trend remained but the differences in toxicity associated with most 5° differences in temperature were insignificant. The difference was significant for rainbow trout, however, at 7 and 12 C. Trout were consistently more sensitive than channel catfish or bluegills.

Water hardness had no effect on toxicity of Noxfish (Tables 2, 3, and 4), with one exception; in 96-h exposures the  $LC_{50}$ 's for channel catfish were 277  $\mu$ g/l in soft water and 328  $\mu$ g/l in very soft water (Table 3). Water at each hardness contained 384 mg/l of sodium bicarbonate to equalize the pH at about 8.0, and that quantity of bicarbonate in the soft water presumably resulted in slightly decreased toxicity to rainbow trout and channel catfish.

The toxicity of Noxfish was not influenced by differences in pH within the range of 6.5 to 9.5 (Tables 2, 3, and 4). Noxfish appeared to be more toxic to rainbow trout at pH 9.5 than at lower pH's, but the increased sensitivity might have been due to an inability of the trout to acclimate fully to the high pH. Bluegills responded uniformly at the three different pH's in soft water at 12 C; the 96-h  $LC_{50}$ 's ranged only from 122 to 138  $\mu$ g/l.

### *Toxicity of Noxfish to Green Eggs of Rainbow Trout*

Newly fertilized eggs of rainbow trout were 47 to 106 times more resistant than fingerlings to Noxfish; the 96-h  $LC_{50}$  ranged from 5.60 mg/l in very soft water to 2.50 mg/l in very hard water (Table 5).

Table 1. Toxicity of Noxfish® to fish in standardized laboratory tests at 12 C.

| Species                             | LC <sub>50</sub> and 95% confidence interval (µg/l) at |           |           |           |
|-------------------------------------|--|-----------|-----------|-----------|
|                                     | 3 h  | 6 h       | 24 h      | 96 h      |
| Bowfin                              | 141  | 106       | 57.5      | 30.0      |
| <i>Amia calva</i>                   | 114-174  | 82.5-136  | 50.4-65.5 | 23.7-38.0 |
| Coho salmon                         | 358  | 152       | 71.6      | 62.0      |
| <i>Oncorhynchus kisutch</i>         | —  | 105-219   | 63.1-81.3 | 54.8-70.2 |
| Chinook salmon                      | 212  | 156       | 49.0      | 36.9      |
| <i>O. tshawytscha</i>               | 171-262  | 137-177   | 44.3-54.2 | 33.9-40.2 |
| Rainbow trout                       | 175  | 86.9      | 68.9      | 46.0      |
| <i>Salmo gairdneri</i>              | 160-191  | —         | 56.2-84.4 | 32.6-64.9 |
| Atlantic salmon                     | 61.5   | 40.0      | 35.0      | 21.5      |
| <i>S. salar</i>                     | 53.4-70.8  | 33.6-70.8 | 29.7-41.2 | 15.5-29.8 |
| Brook trout                         | 141  | 79.7      | 47.0      | 44.3      |
| <i>Salvelinus fontinalis</i>        | 124-160  | 69.2-91.8 | 42.2-52.3 | 41.1-47.7 |
| Lake trout                          | 50.0   | 28.3      | 26.9      | 26.9      |
| <i>S. namaycush</i>                 | 38.6-64.7  | 21.0-38.0 | 19.8-36.5 | 19.8-36.5 |
| Northern pike                       | 181  | 58.2      | 44.9      | 33.0      |
| <i>Esox lucius</i>                  | 160-204  | 52.5-64.5 | 31.4-64.3 | 26.6-41.0 |
| Goldfish                            | —  | —         | —         | 497       |
| <i>Carassius auratus</i>            | —  | —         | —         | 412-600   |
| Carp                                | —  | 270       | 84.0      | 50.0      |
| <i>Cyprinus carpio</i>              | —  | 254-287   | 74.7-94.4 | 41.1-60.8 |
| Fathead minnow                      | —  | 1,190     | 400       | 142       |
| <i>Pimephales promelas</i>          | —  | 917-1,453 | 291-549   | 115-176   |
| Longnose sucker                     | 388  | 218       | 67.2      | 57.0      |
| <i>Catostomus catostomus</i>        | 332-454  | 141-337   | 59.3-76.1 | 51.9-62.6 |
| White sucker                        | 630  | 238       | 71.9      | 68.0      |
| <i>C. commersoni</i>                | 452-878  | 186-304   | 64.0-80.8 | 54.0-85.6 |
| Black bullhead                      | —  | —         | 665       | 389       |
| <i>Ictalurus melas</i>              | —  | —         | 516-856   | 298-507   |
| Channel catfish                     | 1,410  | 840       | 400       | 164       |
| <i>I. punctatus</i>                 | 1,139-1,745  | 717-984   | 234-684   | 138-196   |
| Green sunfish                       | 389  | 332       | 218       | 141       |
| <i>Lepomis cyanellus</i>            | 332-456  | 249-443   | 197-241   | 114-174   |
| Bluegill                            | 424  | 336       | 149       | 141       |
| <i>L. macrochirus</i>               | 335-537  | 245-461   | 124-178   | 133-149   |
| Smallmouth bass                     | 277  | 165       | 93.2      | 79.0      |
| <i>Micropterus dolomieu</i>         | 219-350  | —         | 85.1-102  | 70.7-88.2 |
| Largemouth bass                     | 514  | 360       | 200       | 142       |
| <i>M. salmoides</i>                 | 449-588  | 305-425   | 131-305   | 115-176   |
| Yellow perch                        | 150  | 134       | 92.0      | 70.0      |
| <i>Perca flavescens</i>             | 126-179  | 120-149   | 80.1-106  | 59.8-82.0 |
| Walleye                             | 135  | 52.4      | 16.5      | —         |
| <i>Stizostedion vitreum vitreum</i> | 103-176  | 46.8-58.7 | 15.2-17.9 | —         |

Table 2. *Toxicity of Noxfish® to rainbow trout in water of different temperatures, hardnesses, and pH's.*

| Temp<br>(°C) | Water<br>hardness | pH  | LC <sub>50</sub> and 95% confidence interval (µg/l) at |                   |                   |                   |
|--------------|-------------------|-----|--|-------------------|-------------------|-------------------|
|              |                   |     | 3 h  | 6 h               | 24 h              | 96 h              |
| 7            | Soft              | 7.5 | >400   | 276<br>237-322    | 158<br>134-186    | 70.0<br>62.5-78.4 |
| 12           | Soft              | 7.5 | 175<br>160-191   | 86.9<br>—         | 68.9<br>56.2-84.4 | 46.0<br>32.6-64.9 |
| 17           | Soft              | 7.5 | 73.0<br>59.8-89.1                                      | 73.0<br>59.8-89.1 | 43.4<br>30.9-60.9 | 43.4<br>30.9-60.9 |
| 12           | Very soft         | 8.0 | 122<br>108-138   | 61.9<br>54.9-70.2 | 54.4<br>45.9-64.4 | 54.4<br>45.9-64.4 |
| 12           | Soft              | 8.0 | 90.0<br>81.1-99.9                                      | 62.0<br>51.6-74.5 | 56.5<br>48.2-66.3 | 56.5<br>48.2-66.3 |
| 12           | Hard              | 8.0 | 112<br>95.1-132  | 81.9<br>64.5-104  | 55.1<br>43.9-69.  | 55.1<br>43.9-69.1 |
| 12           | Very hard         | 8.0 | 113<br>92.7-138  | 66.9<br>55.0-81.4 | 53.0<br>44.3-63.4 | 53.0<br>44.3-63.4 |
| 12           | Soft              | 6.5 | 169<br>151-189   | 129<br>107-155    | 78.5<br>69.6-88.6 | 69.5<br>63.7-75.9 |
| 12           | Soft              | 8.5 | 133<br>108-163   | 98.0<br>87.4-110  | 80.0<br>70.3-91.0 | 62.1<br>51.7-74.5 |
| 12           | Soft              | 9.5 | 124<br>106-145   | 75.0<br>63.2-89.0 | 54.0<br>45.9-63.6 | 35.5<br>28.7-43.9 |



Table 3. *Toxicity of Noxfish® to channel catfish at selected temperatures, hardnesses, and pH's.*

| Temp<br>(°C) | Water<br>hardness | pH  | LC <sub>50</sub> and 95% confidence interval ( $\mu\text{g/l}$ ) at |           |         |         |
|--------------|-------------------|-----|---|-----------|---------|---------|
|              |                   |     | 3 h   | 6 h       | 24 h    | 96 h    |
| 12           | Soft              | 7.5 | 1,720   | 1,000     | 539     | 200     |
|              |                   |     | 1,381-2,141   | 784-1,276 | 377-770 | 164-244 |
| 17           | Soft              | 7.5 | 1,410   | 840       | 400     | 164     |
|              |                   |     | 1,139-1,745   | 717-984   | 234-684 | 138-196 |
| 22           | Soft              | 7.5 | 739   | 449       | 164     | 164     |
|              |                   |     | 672-813   | 352-572   | 137-196 | 137-196 |
| 12           | Very soft         | 8.0 | 1,420   | 640       | 476     | 328     |
|              |                   |     | 1,080-1,868   | 464-883   | 346-655 | 290-370 |
| 12           | Soft              | 8.0 | 1,640   | 890       | 450     | 237     |
|              |                   |     | 1,341-2,005   | 677-1,171 | 341-593 | 199-282 |
| 12           | Hard              | 8.0 | 1,220   | 942       | 400     | 308     |
|              |                   |     | 1,023-1,454   | 756-1,173 | 312-513 | 237-400 |
| 12           | Very hard         | 8.0 | 1,160   | 1,000     | 359     | 318     |
|              |                   |     | 996-1,351   | 782-1,279 | 282-457 | 240-421 |
| 12           | Soft              | 6.5 | 1,530   | 865       | 500     | 200     |
|              |                   |     | 1,175-1,991   | 692-1081  | 326-767 | 158-254 |
| 12           | Soft              | 8.5 | 899   | 735       | 565     | 309     |
|              |                   |     | 689-1,173   | 608-889   | 454-704 | 237-403 |
| 12           | Soft              | 9.5 | 629   | 625       | 550     | 248     |
|              |                   |     | 548-721   | 546-715   | 444-681 | 184-335 |

Table 4. *Toxicity of Noxfish® to bluegills at selected temperatures, hardnesses, and pH's.*

| Temp<br>(°C) | Water<br>hardness | pH  | LC <sub>50</sub> and 95% confidence interval ( $\mu\text{g/l}$ ) at |                |                |                |
|--------------|-------------------|-----|---|----------------|----------------|----------------|
|              |                   |     | 3 h   | 6 h            | 24 h           | 96 h           |
| 12           | Soft              | 7.5 | 450<br>353-573  | 270<br>217-335 | 141<br>114-174 | 141<br>114-174 |
| 17           | Soft              | 7.5 | 424<br>335-537  | 336<br>245-461 | 149<br>124-178 | 141<br>133-149 |
| 22           | Soft              | 7.5 | 268<br>240-300  | 227<br>194-266 | 140<br>107-183 | 132<br>122-143 |
| 12           | Very soft         | 8.0 | 334<br>194-575  | 219<br>183-262 | 142<br>129-157 | 132<br>118-147 |
| 12           | Soft              | 8.0 | 450<br>317-639  | 319<br>241-422 | 152<br>135-172 | 137<br>123-153 |
| 12           | Hard              | 8.0 | 300<br>196-460  | 284<br>200-403 | 146<br>131-162 | 132<br>118-147 |
| 12           | Very hard         | 8.0 | 295<br>211-413  | 194<br>148-255 | 138<br>125-152 | 132<br>121-144 |
| 12           | Soft              | 6.5 | 291<br>207-409  | 228<br>194-267 | 150<br>124-181 | 138<br>110-173 |
| 12           | Soft              | 8.5 | 255<br>204-319  | 192<br>170-217 | 122<br>108-138 | 122<br>108-128 |
| 12           | Soft              | 9.5 | 196<br>162-237  | 152<br>134-173 | 122<br>108-138 | 122<br>108-138 |

Although the difference in toxicity was not significant at each hardness increment, the difference was significant in very soft as compared to hard or very hard water.

Table 5. *Toxicity of Noxfish® to newly fertilized eggs of rainbow trout in reconstituted water of different hardnesses at 12 C.*

| Water<br>hardness | 96-h LC <sub>50</sub> (mg/l)<br>and 96% confidence interval |
|-------------------|---|
| Very soft         | 5.60<br>3.55-8.83   |
| Soft              | 4.42<br>3.28-5.96   |
| Hard              | 3.20<br>2.31-4.43   |
| Very hard         | 2.50<br>2.16-2.90   |

### *Persistence of Noxfish in Water*

The toxicity to bluegills of Noxfish solutions aged for 1, 2, and 3 weeks decreased through each week of aging. At 12 C the 96-h LC<sub>50</sub>'s were 133  $\mu\text{g/l}$  in freshly prepared solutions and 254  $\mu\text{g/l}$  in solutions aged for 3 weeks (Table 6). The toxicity decreased

Table 6. *Toxicity (96-h LC<sub>50</sub>'s and 95% confidence intervals in  $\mu\text{g/l}$ ) to bluegills of fresh and aged solutions of Noxfish® in soft water (deactivation indices shown in parentheses).*

| Temp<br>(°C) | Aging time (weeks)         |                           |                          |                          | Half-life<br>(days) |
|--------------|----------------------------|---------------------------|--------------------------|--------------------------|---------------------|
|              | 0                          | 1                         | 2                        | 3                        |                     |
| 12           | 133<br>117-151<br>(1.00)   | —                         | 213<br>193-236<br>(1.60) | 254<br>215-300<br>(1.91) | 22                  |
| 17           | 90.0<br>64.1-126<br>(1.00) | 117<br>92.3-148<br>(1.30) | 190<br>159-228<br>(2.11) | 288<br>247-336<br>(3.20) | 13                  |

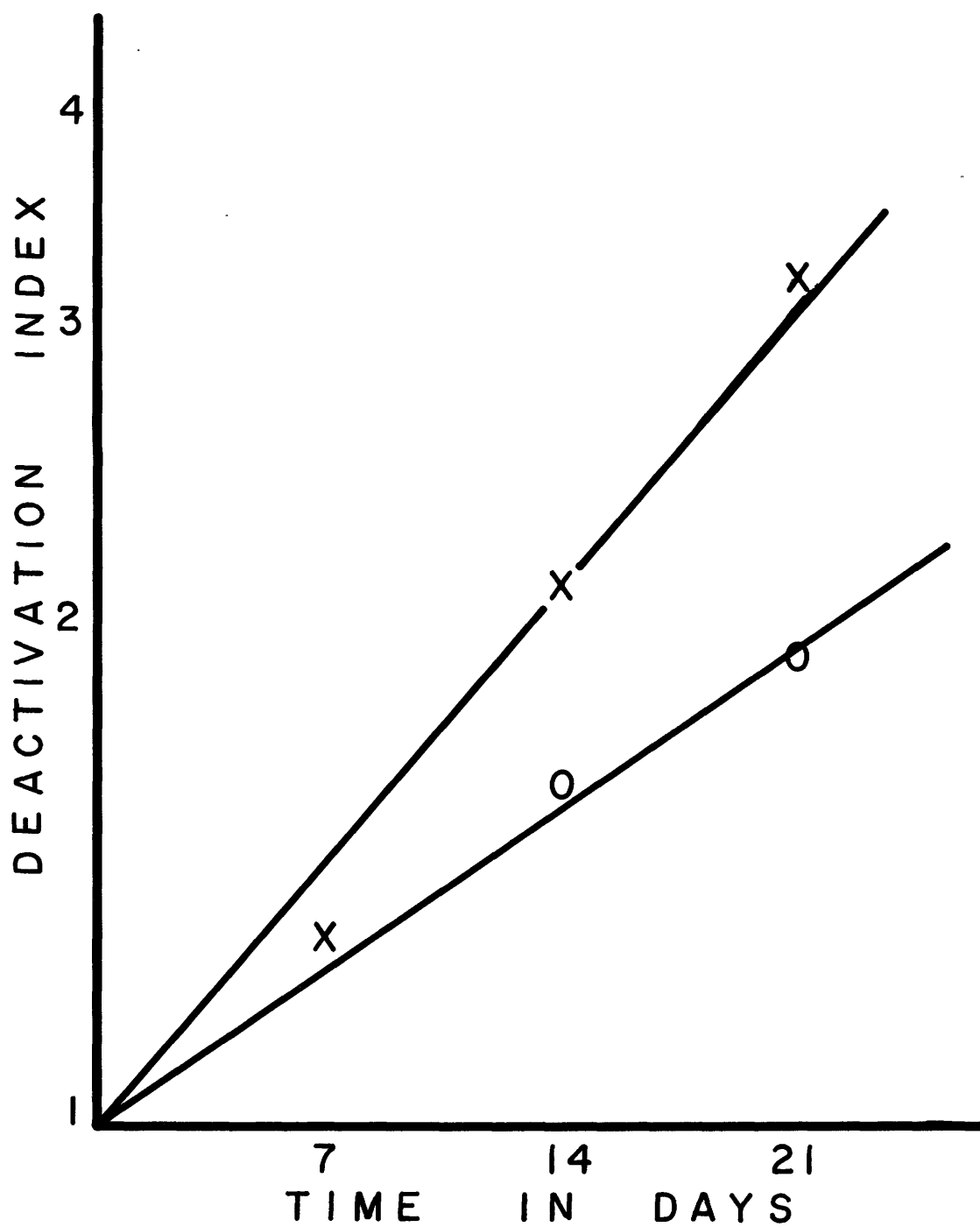


Fig. 1. Detoxification rate for Noxfish® in soft water at 12 C [O] and 17 C [X].

more rapidly at 17 °C than at 12 °C; the half-lives were 13 and 22 days, respectively (Fig. 1).

### *Detoxification of Noxfish*

Static tests with green sunfish showed that potassium permanganate immediately detoxified Noxfish, as indicated by the marked loss of activity in unaged solutions of Noxfish and  $\text{KMnO}_4$  (Table 7). For instance, the 96-h  $\text{LC}_{50}$  at pH 7.5 was 0.241 mg/l for Noxfish alone and 1.71 mg/l for Noxfish plus 1.0 mg/l of  $\text{KMnO}_4$ . The solutions of Noxfish and  $\text{KMnO}_4$  that were aged for 50 min before the fish were added had lost some additional activity (96-h  $\text{LC}_{50}$ , 3.09). The immediate detoxification in unaged solutions was probably a reflection of the effective exposure time, i.e., the time required for Noxfish to produce a lethal effect. The  $\text{KMnO}_4$  detoxified Noxfish in water at all pH's tested.

Chlorine was far less effective than  $\text{KMnO}_4$  for detoxifying Noxfish in laboratory tests. There was little immediate detoxification in unaged solutions and little detoxification during 6 h of aging. For instance, at pH 7.5 the 96-h  $\text{LC}_{50}$  was 0.293 mg/l for Noxfish alone and 0.429 mg/l for Noxfish plus

0.5 mg/l of chlorine (Table 8). In a 6-h aging period, Noxfish was detoxified more efficiently at pH 9.5 than at pH's 7.5 and 8.5.

### *Toxicity of Different Formulations of Rotenone*

Pro-Noxfish, a synergized formulation containing 2.5% rotenone, was more toxic to rainbow trout than the Noxfish formulation or powdered rotenone when concentrations were calculated on the basis of rotenone content. The comparative 96-h  $\text{LC}_{50}$ 's ( $\mu\text{g/l}$ ) were as follows: Pro-Noxfish, 1.02; Noxfish, 3.05; and powdered rotenone, 4.20 (Table 9). There was no significant difference in toxicity between Noxfish and rotenone powder formulations.

### *Toxicity in Flow-through Tests*

In 4-day flow-through exposures, Noxfish was more toxic to chinook salmon and yellow perch than to carp or white suckers (Table 10). Toxicity did not increase with exposure time after 4 days, except for carp (in which toxicity increased through 20 days). Noxfish

Table 7. *Toxicity and detoxification of Noxfish® in static tests with green sunfish in water containing 1.0 mg/l of  $\text{KMnO}_4$  at 12 °C.*

| Compound and (for compounds combined) interaction time (min) <sup>a</sup> | 96-h $\text{LC}_{50}$ and 95% confidence interval (mg/l) at |                      |                      |                      |
|---|---|----------------------|----------------------|----------------------|
|   | pH 6.5  | pH 7.5               | pH 8.5               | pH 9.5               |
| $\text{KMnO}_4$   | 3.47<br>3.12-3.87   | 3.03<br>2.69-3.41    | 1.41<br>1.14-1.74    | 3.08<br>2.32-4.08    |
| Noxfish   | 0.184<br>0.161-0.211  | 0.241<br>0.203-0.287 | 0.158<br>0.114-0.219 | 0.378<br>0.317-0.451 |
| Noxfish + $\text{KMnO}_4$   |   |                      |                      |                      |
| 0   | 1.32<br>1.04-1.68   | 1.71<br>1.49-1.96    | 2.10<br>1.91-2.31    | 1.55<br>1.16-2.08    |
| 10  | 1.17<br>0.948-1.44  | 1.41<br>1.14-1.74    | 1.81<br>1.54-2.13    | 1.36<br>1.07-1.70    |
| 20  | 1.41<br>1.14-1.74   | 2.89<br>2.28-3.36    | 2.38<br>2.24-2.53    | 1.64<br>1.38-1.94    |
| 30  | 1.82<br>1.54-2.15   | 2.89<br>2.28-3.66    | 3.10<br>2.69-3.58    | 1.91<br>1.59-2.29    |
| 40  | 2.00<br>1.64-2.44   | 2.28<br>1.82-2.85    | 3.09<br>2.88-3.32    | 1.41<br>1.14-1.74    |
| 50  | 1.93<br>1.61-2.31   | 3.09<br>2.74-3.49    | 3.59<br>3.19-4.03    | 1.81<br>1.53-2.13    |

<sup>a</sup> Length of time Noxfish and  $\text{KMnO}_4$  were added before fish were introduced.

Table 8. *Toxicity and detoxification of Noxfish® in static tests with green sunfish in water containing 0.5 mg/l of chlorine at 12 C.*

| Compound and (for compounds combined) interaction time (h) <sup>a</sup> | 96-h LC <sub>50</sub> and 95% confidence interval (mg/l) at |                      |                      |
|---|---|----------------------|----------------------|
|   | pH 7.5  | pH 8.5               | pH 9.5               |
| Chlorine  | 0.840<br>0.703-1.00   | 0.820<br>0.588-1.14  | 0.709<br>0.567-0.887 |
| Noxfish   | 0.293<br>0.264-0.325  | 0.338<br>0.304-0.376 | 0.329<br>0.294-0.368 |
| Noxfish + chlorine  |   |                      |                      |
| 0   | 0.429<br>0.359-0.512  | 0.300<br>0.242-0.372 | 0.488<br>0.426-0.559 |
| 0.5   | 0.348<br>0.299-0.405  | 0.492<br>0.427-0.567 | —<br>—               |
| 1.0   | 0.483<br>0.406-0.575  | 0.380<br>0.318-0.455 | 0.900<br>0.677-1.20  |
| 2.0   | 0.412<br>0.337-0.504  | 0.400<br>0.339-0.473 | 0.689<br>0.584-0.813 |
| 4.0   | 0.494<br>0.438-0.557  | 0.489<br>0.432-0.554 | 0.770<br>0.689-0.862 |
| 6.0   | 0.494<br>0.438-0.557  | 0.454<br>0.390-0.529 | 1.37<br>0.988-1.90   |

<sup>a</sup> Length of time Noxfish and chlorine were added before fish were introduced.

Table 9. *Toxicity of three formulations of rotenone to rainbow trout in soft water at 12 C.*

| Preparation       | % active rotenone | LC <sub>50</sub> and 95% confidence interval (μg/l) at |                   |                   |                   |                    |
|-------------------|-------------------|--|-------------------|-------------------|-------------------|--------------------|
|                   |                   | 1 h  | 3 h               | 6 h               | 24 h              | 96 h               |
| Pro-Noxfish®      | 2.5               | 13.0<br>8.15-20.7                                      | 4.53<br>3.28-5.65 | 2.98<br>2.43-3.65 | 1.82<br>1.60-2.08 | 1.02<br>0.917-1.15 |
| Noxfish®          | 5.0               | 25.5<br>16.1-40.5                                      | 8.70<br>6.90-11.0 | 5.50<br>4.87-6.20 | 3.25<br>2.78-3.81 | 3.05<br>2.85-3.27  |
| Powdered rotenone | 33.0              | 16.5<br>14.3-19.1                                      | 8.09<br>6.37-10.3 | 6.60<br>5.41-8.02 | 3.82<br>3.30-4.46 | 3.20<br>2.09-3.70  |

Table 10. *Toxicity of Noxfish® to four species of fish in flow-through toxicity tests at 12 C.*

| Species        | LC <sub>50</sub> and 95% confidence interval ( $\mu\text{g/l}$ ) at |                   |                   |                   |                   |
|----------------|---|-------------------|-------------------|-------------------|-------------------|
|                | 1 day   | 4 days            | 10 days           | 20 days           | 30 days           |
| Chinook salmon | 112<br>97.7-128   | 71.0<br>55.3-99.1 | 62.0<br>52.1-73.7 | 59.0<br>49.5-70.3 | —                 |
| Carp           | —   | 142<br>122-165    | 96.0<br>78.0-118  | 67.0<br>57.4-78.3 | 68.0<br>57.7-80.1 |
| White sucker   | —   | 144<br>122-170    | 129<br>118-141    | 112<br>95.5-131   | 112<br>95.5-131   |
| Yellow perch   | 160<br>121-211  | 60.0<br>53.3-67.6 | 50.0<br>36.8-68.0 | 46.0<br>32.7-64.8 |                   |

was consistently and significantly more toxic to all four species in static than in flow-through tests (Table 11).

## Discussion

The literature on toxicity of rotenone to fish suggests that concentrations used in fishery management are generally higher than those known to be lethal in laboratory tests; that toxicity depends on temperature, water hardness, pH, and physical characteristics; and that many different application rates may be effective for the same target species of fish (Schnick 1974; Meyer 1966).

Since laboratory procedures are usually more standardized than field procedures, laboratory data are expected to be more consistent than field data. Although the LC<sub>50</sub>'s of less than 0.2 mg/l for

Noxfish against rainbow trout, channel catfish, and bluegills reported by Bridges and Cope (1965) were similar to ours, applications of at least 1 mg/l have been repeatedly recommended for eliminating these species. Spitler (1970) reported that 1.6 mg/l of Noxfish was not effective and that as much as 5 mg/l was sometimes needed. The difference in laboratory and field data is due to several factors. Laboratory data generally indicate concentrations that produce 50% mortality (LC<sub>50</sub>), whereas field concentrations are based on eliminating 100% of the target fish. Organisms, particulate matter, and sunlight contribute to the tendency toward faster detoxification of chemicals in natural waters than in the laboratory. Furthermore, because uniform concentrations are much more difficult to obtain in the field, additional amounts of toxicants are generally applied to ensure a lethal concentration throughout a body of water.

Although some of the reports are conflicting, rotenone is generally more effective at high than at low temperatures (Gersdorff 1943; Almquist 1959; Ball 1948; Hooper 1955), in acid than in alkaline waters (Leonard 1939; Foye 1964), and in soft than in hard water (Foye 1964). In many of these studies, however, efficacy was based on survival time of the fish rather than on the concentration of the toxicant. Our laboratory data show only slight changes in the toxicity of rotenone at different temperatures, hardnesses, or pH's. Consequently, concentrations used in the field should be based on the results of on-site toxicity tests (Burruss 1975) rather than on extrapolations of laboratory or field data.

Most studies—in laboratory or field—have shown that goldfish and black bullheads are the species most resistant to rotenone. Individual fish of a species may be exceptionally resistant (Meyer 1966)—an observation that may explain some incomplete fish kills and the need to apply a concentration

Table 11. *Comparison of acute toxicities of Noxfish® to four species of fish in 96-h flow-through and static tests in carbon filtered municipal water at 12 C.*

| Species        | LC <sub>50</sub> and 95% confidence interval ( $\mu\text{g/l}$ ) |                   |
|----------------|--|-------------------|
|                | Static   | Flow-through      |
| Chinook salmon | 34.7<br>26.9-44.7  | 71.0<br>55.3-99.1 |
| Carp           | 19.0<br>12.1-29.9  | 142<br>122-165    |
| White sucker   | 17.9<br>12.8-25.1  | 144<br>122-170    |
| Yellow perch   | 30.0<br>23.6-38.2  | 60.0<br>53.3-67.6 |

greater than that indicated in laboratory tests.

The detoxifiers  $\text{KMnO}_4$  and chlorine were toxic to fish at concentrations only slightly greater than those needed to detoxify rotenone. For example, against green sunfish in water at pH 8.5, the 96-h  $\text{LC}_{50}$  for  $\text{KMnO}_4$  was 1.41 mg/l and that for chlorine was 0.82 mg/l. These results support Engstrom-Heg and Loeb (1968) and Engstrom-Heg (1972), who cautioned that high concentrations of  $\text{KMnO}_4$  may become toxic and may have to be reduced with sodium thiosulfate or other agents. Therefore, detoxifiers should be used only when necessary and in only the quantities needed.

## References

- Almquist, E. 1959. Observations on the effect of rotenone emulsives on fish food organisms. Inst. Freshwater Res. Drottningholm Rep. 40:146-160.
- Baker, P. H., and A. J. Cordone. 1969. Distribution, size composition, and relative abundance of the Piute sculpin, *Cottus beldingii* Eigenmann and Eigenmann, in Lake Tahoe. Calif. Fish Game 55(4):285-297.
- Ball, R. C. 1948. A summary of experiments in Michigan lakes on the elimination of fish populations with rotenone, 1934-1942. Trans. Am. Fish. Soc. 75(1945):139-146.
- Bridges, W. R., and O. B. Cope. 1965. The relative toxicities of similar formulations of pyrethrum and rotenone to fish and immature stoneflies. Pyrethrum Post 8(1):3-5.
- Burruss, R. M. 1975. Development and evaluation of on-site toxicity test procedures for fishery investigations. U.S. Fish Wildl. Serv. Invest. Fish Control 68. 8 pp.
- Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecol. Res. Ser. EPA [Environ. Prot. Agency]-660/3-75-009. 61 pp.
- Engstrom-Heg, R. 1972. Kinetics of rotenone-potassium permanganate reactions as applied to the protection of trout streams. N.Y. Fish Game J. 19(1):47-58.
- Engstrom-Heg, R., and H. A. Loeb. 1968. Chemical detoxification of rotenone: a simple case of oxidation? N.Y. Conserv. Dep., Bur. Fish, N.Y. Fish News 27:3-7.
- Foye, R. E. 1964. Chemical reclamation of forty-eight ponds in Maine. Prog. Fish-Cult. 26(4):181-185.
- Gersdorff, W. A. 1943. Effect of change of temperature on relative toxicity of rotenone and phenol. J. Agric. Res. 67(2):65-80.
- Hooper, F. F. 1955. Eradication of fish by chemical treatment. Mich. Dep. Conserv., Fish Div. Pam. No. 19. 6 pp.
- Hunn, J. B., R. A. Schoettger, and E. W. Whealdon. 1968. Observations on the handling and maintenance of bioassay fish. Prog. Fish-Cult. 30(3):164-167.
- Lennon, R. E. 1967. Clearance and registration of chemical tools for fisheries. Prog. Fish-Cult. 29(4):187-193.
- Lennon, R. E., and C. R. Walker. 1964. Laboratories and methods for screening fish-control chemicals. U.S. Fish Wildl. Serv. Invest. Fish Control 1 (Circ. 185). 15 pp.
- Lennon, R. E., J. B. Hunn, R. A. Schnick, and R. M. Burruss. 1970. Reclamation of ponds, lakes, and streams with fish toxicants: a review. FAO Fish. Tech. Pap. No. 100. 99 pp.
- Leonard, J. W. 1939. Notes on the use of derris as a fish poison. Trans. Am. Fish. Soc. 68(1938):269-279.
- Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96(2):99-113.
- Marking, L. L. 1969. Toxicological assays with fish. Bull. Wildl. Dis. Assoc. 5:291-294.
- Marking, L. L. 1972. Methods of estimating the half-life of biological activity of toxic chemicals in water. U.S. Fish Wildl. Serv. Invest. Fish Control 46. 9 pp.
- Marking, L. L. 1975. Toxicological protocol for the development of piscicides. Pages 26-31 in P. H. Eschmeyer, ed. Rehabilitation of fish populations with toxicants: a symposium. Am. Fish. Soc., North Central Div., Spec. Publ. No. 4.
- Marking, L. L., and T. D. Bills. 1975. Toxicity of potassium permanganate to fish and its effectiveness for detoxifying antimycin. Trans. Am. Fish. Soc. 104(3):579-583.
- Meyer, F. A. 1966. Chemical control of undesirable fishes. Pages 498-510 in A. Calhoun, ed. Inland fisheries management. Calif. Dep. Fish Game, Sacramento.
- Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. Water Res. 1:21-29.
- Olson, L. E., and L. L. Marking. 1973. Toxicity of TFM (lambpricide) to six early life stages of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30:1047-1052.
- Schnick, R. A. 1974. A review of the literature on use of rotenone in fisheries. U.S. Fish Wildl. Serv. Lit. Rev. 74-15. NTIS [Natl. Tech. Inf. Serv.] No. PB-235 454/AS. 130 pp.
- Spitler, R. J. 1970. An analysis of rotenone treatments for estimation of fish populations in southern Michigan lakes, 1957-1967. Mich. Acad. 3(1):77-82.









(Reports 56 through 59 are in one cover.)

- 56. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to 10 Species of Algae, by A. A. Maki, L. D. Geissel, and H. E. Johnson. 1975. 17 pp.
- 57. Acute Toxicities of 3-Trifluoromethyl-4-nitrophenol (TFM) and 2',5-Dichloro-4'-nitrosalicylanilide (Bayer 73) to Larvae of the Midge *Chironomus tentans*, by J. A. Kawatski, M. M. Ledvina, and C. R. Hansen, 1975. 7 pp.
- 58. Acute Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nymphs of Mayflies (*Hexagenia* sp.), by C. R. Fremling. 1975. 8 pp.
- 59. Toxicity and Residue Dynamics of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) in Aquatic Invertebrates, by H. O. Sanders and D. F. Walsh. 1975. 9 pp.

(Reports 60 through 62 are in one cover.)

- 60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests, by L. L. Marking and L. E. Olson. 1975. 27 pp.
- 61. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Flow-Through Tests, by L. L. Marking, T. D. Bills, and J. H. Chandler. 1975. 9 pp.
- 62. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Selected Aquatic Invertebrates and Frog Larvae, by J. H. Chandler and L. L. Marking. 1975. 7 pp.

(Reports 63 through 66 are in one cover.)

- 63. Laboratory Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM) as a Lampricide, by V. K. Dawson, K. B. Cumming, and P. A. Gilderhus. 1975. 7 pp.
- 64. Effects of 3-Trifluoromethyl-4-nitrophenol (TFM) on Developmental Stages of the Sea Lamprey, by G. W. Piavis and J. H. Howell. 1975. 4 pp.
- 65. Accumulation and Loss of Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in Fish Muscle Tissue; Laboratory Studies, by J. B. Sills and J. L. Allen. 1975. 5 pp.
- 66. Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in a Stream Ecosystem after Treatment for Control of Sea Lampreys, by P. A. Gilderhus, J. B. Sills, and J. L. Allen. 1975. 5 pp.
- 67. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals, by L. L. Marking and V. K. Dawson. 1975. 7 pp.
- 68. Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations, by R. M. Burress. 1975. 8 pp.

(Reports 69 and 70 are in one cover.)

- 69. Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM), 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture to Fingerlings of Seven Fish Species and to Eggs and Fry of Coho Salmon, by T. D. Bills and L. L. Marking. 1976. 9 pp.
- 70. The Freshwater Mussel (*Anodonta* sp.) as an Indicator of Environmental Levels of 3-trifluoromethyl-4-nitrophenol (TFM), by A. W. Maki and H. E. Johnson. 1976. 5 pp.
- 71. Field Tests of Isobornyl Thiocynoacetate (Thanite) for Live Collection of Fishes, by R. M. Burress, P. A. Gilderhus, and K. B. Cumming. 1976. 13 pp.

Fish Control Laboratories  
Fish and Wildlife Service  
U.S. Department of the Interior  
P.O. Box 862  
La Crosse, Wisconsin 54601

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the wisest use of our land and water resources, protecting our fish and wildlife, preserving the environmental and cultural values of our national parks and historical places, and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to assure that their development is in the best interests of all our people. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.



GPO 836-853

FISH CONTROL LABORATORIES  
U.S. FISH AND WILDLIFE SERVICE  
P.O. BOX 862  
LA CROSSE, WISCONSIN 54601

POSTAGE AND FEES PAID  
U.S. DEPARTMENT OF THE INTERIOR

INT 423

