INVESTIGATIONS IN FISH CONTROL

68. Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations

United States Department of the Interior
Fish and Wildlife Service

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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.

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68. Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations

By Ralph M. Burress

United States Department of the Interior
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CONTENTS

DEVELOPMENT AND EVALUATION OF ON-SITE TOXICITY TEST PROCEDURES FOR FISHERY INVESTIGATIONS

by

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ABSTRACT

A simple, inexpensive procedure was developed for conducting on-site tests (bioassays) of the toxicity of various concentrations of antimycin on target and nontarget fishes in waters to be treated. After preliminary laboratory experiments with seven types of containers showed that large polyethylene bags were best for use in field toxicity tests, year-round field experiments were performed in ponds. Measurements of water quality in large bags containing 284 liters of water were closely similar to water quality measurements in ponds throughout the test periods. The maximum safe loading level for 96-h tests at temperatures above 18.3 C was about 852 g of fish per bag, but this amount could be increased in colder water or in shorter tests. Final modifications and evaluations of the method were made on the basis of treatments of ponds with antimycin in accordance with data derived from on-site tests. The procedure described is adaptable for tests of many other chemical compounds commonly used by fishery workers.

INTRODUCTION

With the development of more potent and more selective fish toxicants, fishery managers have an increasing need to determine the concentration of toxicant which is most effective for a specific purpose in a body of water. The efficacy of chemicals used by fishery workers is affected by water quality, physical and biological conditions in the aquatic habitat, and the relative susceptibility of different kinds and sizes of target organisms. The lack of specific information on which to base application rates has often resulted in inadvertent underdosing, deliberate overdosing, and repeated dosing, all of which were detrimental to the success of the treatments or to the habitats treated.

The on-site toxicity test (bioassay) procedure is one of the more promising methods for determining both the concentration and the length of exposure required to produce the desired level of control of target organisms. Despite the pronounced need for information of this kind, the on-site test method apparently has not been widely used; relatively few investigators who have applied toxicants to manipulate fish populations in streams have reported using fish and water from the streams to be treated to conduct preliminary on-site tests. The facilities employed have varied from a simple arrangement of fish hatchery troughs beside a stream (Lennon and Parker 1959) to a

fully equipped mobile laboratory (Howell and Marquette 1962). On-site tests conducted in lakes before the application of fish toxicants have been performed in large wastebaskets (Berger et al. 1967), in 91-liter plastic garbage cans (Pfeiffer 1968), in open-ended, 208-liter barrels that were pushed into the lake bottom a few inches (Vaughn et al. 1974), and in 76-liter capacity plastic bags that were included in kits provided by Ayerst Laboratory Inc., for on-site tests of antimycin (G. C. Radonski, personal communication).

The utility and economy of using small plastic bags as bioassay vessels are well documented. Davis and Hardcastle (1959) used 25-liter bags enclosed in cardboard cartons to conduct herbicide toxicity tests in the laboratory; Falk (1972) conducted acute toxicity bioassays of pollutants by placing 20-liter plastic bags inside larger nylon mesh bags supported by rigid aluminum frames situated in shallow lake waters. In both these studies, the authors placed 10 fish in each bag and supplied continuous aeration to maintain adequate concentrations of dissolved oxygen for 48 or 96 h.

Although dissolved oxygen diffuses through polyethylene film (Fremling and Evans 1963), the rate of diffusion is so slow that, if small plastic bags are used as bioassay containers, either accessory aeration must be supplied or the loading levels (grams of fish per liter of water) must be light and tests must be of short duration. In the present study, the objective was to use more and larger fish in 96-h tests of antimycin without employing either aeration devices or bulky supports for rigid vessels. Consequently, the study centered on determining the feasibility of employing plastic bags large enough to contain 284 liters of water and on developing simple experimental procedures for their use. During the period July 1968 to June 1970, numerous laboratory and field experiments were conducted at the Southeastern Fish Control Laboratory, Warm Springs, Georgia and in four nearby ponds. Bioassay procedures developed were tested in field trials in Illinois and Arkansas in 1972.

MATERIALS AND METHODS

Laboratory experiments included comparisons of the utility, efficacy, cost, and safety of seven types of potential on-site test vessels: polyethylene wastebaskets, fiber glass containers, metal lard cans, aluminum pails, stainless steel pails, large glass jars, and polyethylene bags. No single container was superior to the others in every respect, but polyethylene bags appeared to be the most useful, primarily because they are readily portable and are available in large sizes from numerous firms that manufacture plastic film or plastic containers. Consequently, large polyethylene bags (0.96 m × 1.65 m, made of material 0.076 mm [3 mils] thick) were used in conducting 19 on-site toxicity tests with antimycin in four ponds during the period April 1969 to June 1970. The ponds varied in type from a clear, infertile pond to a highly eutrophic pond polluted with dairy wastes. Loading rates ranged from 0.11 to 2.76 g/l, but were less than 1 g/l in 13 of the tests. Certain procedures were modified in the exploratory stage of the study, but the methods outlined here were followed consistently thereafter.

Experimental Fish

In most experiments, antimycin was tested against bluegills (*Lepomis macrochirus*); in others, a few subadult largemouth bass (*Micropterus salmoides*) or channel catfish (*Ictalurus punctatus*) were included. Most of the fish were seined from the ponds in which the tests were conducted. These fish, usually captured a day or two before the tests, were held in uncrowded live-cages in the ponds while they recovered from the stress of collection, and only vigorous specimens were selected for testing. The few fish from the laboratory that were used in field tests also were held at least 24 h in live-cages for acclimation to pond conditions. If the supply of fish was adequate, 10 fish were placed in each vessel except when the use of large fish made it necessary to reduce the number to avoid exceeding the loading capacity.

Preparation of Stock Solutions

Stock solutions were prepared by using a 1-ml glass pipette graduated in hundredths to

measure 1.42 ml of Ayerst's undiluted 20% stock solution of antimycin [Fintrol-Concentrate (R)] into a 1-liter volumetric flask and adding acetone to bring the volume up to 1 liter. The addition of 1 ml of this stock solution to 284 liters of water yielded a 1-μg/l (1-ppb) concentration of antimycin (active ingredient), a procedure which simplified operations and reduced the likelihood of error. The small amounts of toxicant needed for determining the concentrations required for selective kills were also measured with a 1-ml glass pipette.

On-site Toxicity Test Arrangement

In selecting the site, the crew avoided areas where rocks, roots, or other objects might puncture the bags. Where no protective enclosure for the bags was needed, two steel fence posts were driven into the bottom of the pond about 7 m apart in waist-deep water, a rope was stretched between the posts, and the bags were suspended from the rope with stout twine (Fig. 1). When necessary, bags were protected from sources of mechanical damage (e.g., turtles, boats, and flotsam) by making a simple exclosure of netting material supported by four steel fence posts. The ropes used to support the bags were tied diagonally across the exclosure, providing support for a cover made of netting and eliminating bird depredations that sometimes occurred when treated fish surfaced in distress.

Filling Bags

In the laboratory, 284 liters of water were pumped into a bag and the water level was marked. The bag was then emptied, and about 50 more bags were marked at the same level for use in the field. Two men standing in waist-deep water were able to fill and handle bags without difficulty. Water of this depth permits crewmen in chest waders to handle full bags without dragging them over the pond bottom. The preferred method for filling bags involved the use of a large plastic waste can from which the bottom had been removed. The can, when fully inserted into the mouth of the bag, held the bag open and gave good support as the bag was filled. When water reached the desired level, the can was removed, the bag neck was twisted shut and secured with a stout rubber band, and the bag was suspended from the rope with twine. After all bags were filled, each was reopened; the toxicant was added and mixed into the water thoroughly with a dip net; fish were added; the bag was resealed, returned to its place, and numbered. In each experiment, two controls (untreated) were used; one contained fish and the other only water.

Observations

In clear ponds dead fish could be seen at the bottom of the bags and removed easily with a dip net when observations were made. If the water was not clear enough to permit direct observation, all fish were dipped out into a container. Live fish then were returned to the bags; dead fish were discarded. Use of a rectangular dip net expedited removal of dead fish from the corners of bags.

Monitoring water quality

Most tests were conducted for 96 h. Measurements of pH and dissolved oxygen in the pond, in the control bag containing fish, and in the control bag without fish, generally were made in early morning and late afternoon. A two-man crew could set up and conduct a test without undue difficulty if work schedules were efficiently arranged.

EFFICACY OF POLYETHYLENE BAGS FOR USE IN ON-SITE TOXICITY TESTS

The results of the brief initial experiments with large polyethylene bags were encouraging. Bags of 0.076-mm wall thickness proved to be sufficiently strong. Furthermore, struggling fish in their death throes invariably retracted their fins when they came in contact with the bag wall and thus did not puncture the bags. If seams leaked or the bag was punctured, the medium was not diluted because the water flow always was outward.

Results of routine static tests conducted to compare the toxicity of antimycin to fingerling bluegills in glass jars and in plastic bags were closely comparable; this indicates that no

appreciable loss of toxicant resulted from absorption, adsorption, or reaction with the plastic. Before pond tests were begun, other tests were performed in outdoor plastic pools containing phytoplankton. Water temperatures in the bags were virtually identical with those at corresponding depths outside the bags, and the pH of water in the bags corresponded more closely with that in the surrounding water than did the pH in other types of containers. Concentrations of dissolved oxygen remained higher in clear bags than in opaque bags, presumably because photosynthesis continued at a higher rate in clear bags.

The influence of 2-, 3-, 4-, and 5-g/l loading rates on pH and dissolved oxygen concentrations were determined in a summer field test. When surface temperatures were high (28.0–31.9 C), the 3-g/l loading with bluegills of intermediate size was the highest that could be used for a 96-h test. A 4-g/l load could have been used for as long as 48 h, but a 5-g/l load was too great for even a 24-h test.

Comparing pH values and dissolved oxygen concentrations in bags and various types of ponds during different seasons was a primary concern during the development and evaluation of toxicity test procedures. Water quality data collected during the 19 field tests indicated that the highest and lowest pH values measured in the control bags containing fish were neither consistently higher nor lower than those measured at the pond surface (Table 1). Thus, test results were not biased by differences in pH attributable to the bags. Comparisons of dissolved oxygen concentrations showed that average readings in the control bags containing fish tended to be slightly lower than those in the pond, whereas the readings in the control bags without fish generally were somewhat higher. However, no test results were invalidated because of oxygen depletion in the bags. If bags made from different grades of raw materials have different physical and chemical characteristics, oxygen may diffuse less readily through some bags than others. Preliminary tests with each new lot of bags would help determine safe loading rates.

After the 19 field trials were completed and basic test methods were established, on-site toxicity tests were conducted in one pond in Illinois and four in Arkansas. The antimycin treatments were successful, and were reported by Cumming and Gilderhus (in press) and by Cumming, Burress, and Gilderhus (1975). Properly secured bags withstood heavy rains, winds, and even strong currents induced by outboard motor operation. There is good reason to believe that such tests also could be conducted in streams with a slow or moderate current, if bags were protected from floating objects. Because of the essentially neutral buoyancy of the bags, it is possible to anchor them and conduct tests below the surface. G. C. Radonski (personal communication) stated that he has conducted toxicity tests in plastic bags under ice and in the hypolimnion at depths as great as 9.1 m.

RECOMMENDATIONS FOR CONDUCTING ON-SITE TESTS

The methods outlined above provide the basic guidelines for conducting an on-site toxicity test. The first day's preparations include securing the experimental fish; setting up the posts, ropes, and protective netting; and determining pH and other water quality factors. On the day of the test, the bags are filled, the toxicant is added and thoroughly mixed, and the fish are added in rapid sequence at the time of day when the full-scale treatment will be applied. This ensures that water quality conditions during the test and the treatment will be as similar as possible.

Selecting Test Concentrations

The range of toxicant concentrations to be tested depends largely on the size and relative susceptibility of the target fishes, the amount of population reduction desired, and the water quality characteristics (particularly temperature and pH). Not less than five test concentrations should be used, and a sixth bag, with fish, should be used as a control. The following concentrations of antimycin (μg/l) are suggested when total kills of susceptible species are desired: 2.5, 4.0, 5.5, 7.0, and 8.5. If the

target fishes include resistant species such as goldfish (*Carassius auratus*), gar (*Lepisosteus* spp.), or bowfin (*Amia calva*), concentrations of 12, 15, 18, 21, and 24 μg/l are more appropriate. Less antimycin is required when the temperature is above 15.6 C or the pH is below 8.5.

Selective reduction of target species by complete treatment of the aquatic system requires much lower concentrations of antimycin. When the temperature exceeds 15.6 C and there are marked diurnal fluctuations in pH, the following test concentrations of antimycin (μg/l) are suggested: 0.20, 0.35, 0.50, 0.75, and 1.0. If pH levels are so high that the 1.0-μg/l concentration is not adequate, the range of concentrations should be shifted upward or the treatment postponed until water conditions become more favorable. The following concentrations (μg/l) are more appropriate for use at temperatures lower than 15.6 C: 0.4, 0.8, 1.2, 1.6, and 2.0. The duration of toxicity tests should be extended by 1 to 3 days when water temperatures are low.

Test Animals

In tests where the biologist must determine the ability of fish to withstand exposure to a toxicant in order to ensure selection of a safe concentration for treatment (e.g., removal of scalefish from a catfish pond), use of fish from the pond to be treated is mandatory. In other situations as well, test animals from the waters to be treated should be used if they can be collected in good condition and in adequate numbers. If this is not possible, target and nontarget fishes of appropriate sizes must be brought in from other sources and allowed to acclimate for 24 h in live-cages before the test. Sizes and numbers needed depend on the loading levels that can be used under conditions existing at the time of the test.

Loading Rates

The following information provides broad guidelines regarding loading rates. At temperatures of 25 to 30 C, up to a 3-g/l load (852 g per bag) of fish can be used for 96-h tests. For each 5-degree reduction in temperature below 25 C, it may be possible to increase the loading by 1 g/l (284 g per bag). For 24-h tests, these amounts can be increased, depending upon the circumstances. If excessive loading rates are used, results of the test are likely to be too biased to be usable. Temperature, dissolved oxygen, and pH should be measured each morning and afternoon throughout the test period.

Duration of Toxicity Tests

Test results are apparent much sooner at high than at low temperatures, and the concentration of antimycin required for a complete kill in warm weather can safely be selected on the basis of a 24-h test. In cold weather such tests should be conducted for at least 48 h to avoid the use of unnecessarily high concentrations of toxicant.

Selection of concentrations to effect partial kills can be based on 24-h tests only if temperature and pH are high enough to ensure complete detoxification of the antimycin within that time. When temperatures are low and the pH is neutral or acid, tests for partial kills should be carried out for 96 h or until the full effects of the various concentrations are discernible.

Selecting Treatment Concentrations

The results of on-site toxicity tests can be used in selecting treatment concentrations if two conditions are met: (1) mortality patterns must be consistent, i.e., mortalities must increase as toxicant concentrations increase, and (2) mortality of the control fish must not exceed 10%. Inconsistent patterns of mortality often are a result of using poor quality test animals. If test results are good, the lowest concentration at which all target fish are killed should be used when the entire volume of water is treated to effect a selective kill. The concentration of toxicant used in each bag can be calculated precisely because the volume of water is known. Success in applying test results to actual treatments is directly proportional to the accuracy with which the volume of water to be treated is determined.

When a complete kill is intended and large specimens of the target species are used in the toxicity test, the lowest completely effective concentration tested should be increased by at least 1.0 μg/l to ensure effectiveness of the treatment. If large specimens are not used, a safety factor of at least 1.5 to 3.0 μg/l should be added to the lowest completely effective bioassay concentration, depending on the susceptibility of the target species.

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Table 1.—Temperature, pH, and dissolved oxygen ranges in ponds where on-site bioassay experiments were conducted.

Date	Test period (h)	Loading level (g/l)	Surface temperature range (°C)	pH range			Dissolved oxygen range (mg/l)		
					Plastic bags			Plastic bags	
				Pond	Fish	No fish	Pond	Fish	No fish
4/69	99	0.75	17.8-23.4	6.7- 8.2	6.8- 8.0	7.2- 8.1	6.6-10.0	6.0- 9.5	7.5- 9.5
5/69	70	0.93	21.7-30.6	6.7- 8.2	6.3- 6.8	6.7- 7.3	5.4- 9.6	0.5- 6.5	4.9- 8.2
5/69	70	0.68	18.9-27.2	6.8- 8.4	6.9- 8.5	7.2- 8.7	6.3-10.5	5.7- 9.2	5.9-10.3
5/69	107	0.45	22.2-30.0	6.7- 8.9	6.6- 7.6	6.6- 8.9	6.1-13.0	3.3-10.4	5.8-14.3
5/69	83	0.55	23.4-30.6	6.6- 9.3	7.2- 9.4	7.8- 9.4	5.4-13.5	8.2-14.1	7.2-15.4
6/69	72	0.31	29.5-35.0	7.3- 8.9	7.2- 9.0	7.8- 9.1	6.8-10.7	7.5-10.3	7.7-10.8
7/69	72	a	26.1-31.7	7.6-10.3	7.9-10.4	7.9-10.3	3.0-14.0	6.2-24.0	8.6-19.9
8/69	72	1.41	26.7-31.1	6.6- 8.3	6.6- 8.5	6.6- 8.3	6.1- 9.0	5.2- 8.7	6.5- 9.0
11/69	96	0.46	13.7-16.2	5.9- 6.4	5.1- 6.6	6.1- 6.5	8.8- 9.8	9.2-10.0	9.6-10.0
11/69	96	1.23	13.7-16.2	5.9- 6.4	5.5- 6.1	6.1- 6.5	8.8- 9.8	5.0-10.6	9.6-10.0
11/69	120	0.62	11.2-14.5	5.8- 6.3	6.3- 6.8	6.3- 6.8	7.4-10.0	7.8- 9.0	8.6-10.4
11/69	120	1.24	11.2-14.5	5.8- 6.3	6.0- 6.2	6.3- 6.8	7.4-10.0	4.7- 6.3	8.6-10.4
11/69	72	0.99	8.9-12.2	7.0- 7.3	6.6- 7.0	7.2- 7.4	10.2-13.3	10.4-11.2	10.8-12.8
12/69	72	0.89	6.8- 8.9	7.3- 7.6	7.3- 7.4	7.3- 7.4	10.0-14.0	12.6-13.2	13.5-13.6
1/70	81	2.76	8.9-15.6	7.3- 8.8	6.8- 8.6	—	11.4-14.5	8.6-13.8	—
3/70	96	0.85	12.2-15.6	5.3- 6.4	5.9- 6.1	6.0- 6.1	5.0- 8.2	—	—
4/70	96	0.11	17.8-21.1	6.8- 7.5	6.8- 7.5	6.8- 7.2	6.6-10.4	8.6-10.4	8.4-10.5
6/70	96	0.49	23.4-26.1	7.5- 8.9	7.4- 8.7	7.7- 8.8	8.6-12.2	8.0-12.4	9.4-12.2
6/70	96	0.55	25.0-31.7	7.0- 9.3	7.0- 8.6	7.0- 8.3	6.2-11.2	8.5- 9.8	8.1-11.2

[a] Turtles damaged bag, allowing fish to escape.

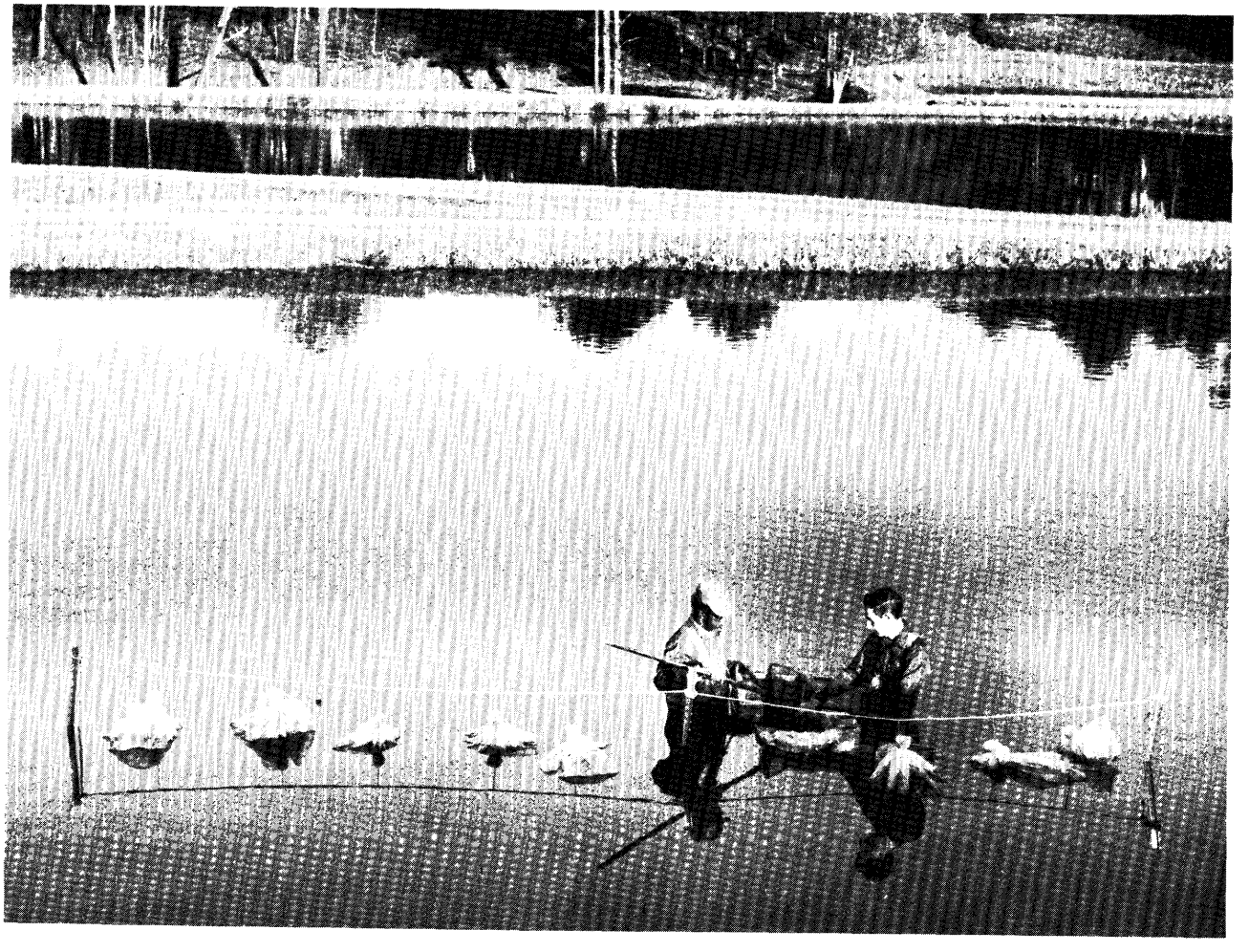

Figure 1. Sample apparatus for suspending large plastic bags used to conduct on-site toxicity tests.

(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.

(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.
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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.
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(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.
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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.

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