INVESTIGATIONS IN FISH CONTROL

53. Toxicity of Mixtures of Quinaldine Sulfate and MS-222 to Fish

54. The Efficacy of Quinaldine Sulfate:MS-222 Mixtures for the Anesthetization of Freshwater Fish

55. Residues of Quinaldine and MS -222 in Fish Following Anesthesia with Mixtures of Quinaldine Sulfate:MS-222

United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

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52. Residues of MS-222, Benzocaine, and Their Metabolites in Striped Bass Following Anesthesia, by Charles W. Luhning. 1973. 11 p.

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IN THIS COVER

FOREWORD

All chemicals, including anesthetics, which are to be used on food or game fishes must be approved and registered by the Food and Drug Administration. Research to support petitions for registration of such compounds is an integral part of the program of the Fish Control Laboratories. Studies involving anesthetics have centered on quinaldine and tricaine methanesulfonate (MS-222, Finquel(R)), the two most commonly used anesthetics for fish.

While each compound is effective in itself, studies have shown that when MS-222 and quinaldine are used together, desired anesthesia is achieved without undesirable side effects noted when the compounds are used singly.

Registration-oriented research on MS-222 was reported in Investigations in Fish Control (IFC), numbers 12-17. The development of a water-soluble salt of quinaldine and related studies to support a petition for its registration are found in IFC, numbers 47-50.

The papers which follow are concerned with research on the toxicity, efficacy, and residues associated with the use of mixtures of quinaldine sulfate and MS-222 as an anesthetic for selected coldwater and warmwater fishes. The data presented will be used to support a petition for registration to permit the use of such mixtures on fish.

Fred P. Meyer, Director
Fish Control Laboratories

INVESTIGATIONS IN FISH CONTROL

53. Toxicity of Mixtures of Quinaldine Sulfate and MS-222 to Fish

By Verdel K. Dawson and Leif L. Marking

United States Department of the Interior
Fish and Wildlife Service
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CONTENTS

TOXICITY OF MIXTURES OF QUINALDINE SULFATE AND MS-222 TO FISH

By Verdel K. Dawson and Leif L. Marking
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ABSTRACT.--The acute toxicities of mixtures of two fish anesthetics (quinaldine sulfate and MS-222) to coho salmon, rainbow trout, brown trout, brook trout, lake trout, carp, channel catfish, bluegill, and largemouth bass of various sizes were determined in 15-, 30-, and 60-minute and 24-, 48-, and 96-hour static toxicity tests. The effects of various temperatures, water hardnesses, and pH's on the mixture's toxicity were evaluated. The 96-hour LC50's of $QdSO_4$:MS-222 in ratios of 1:4 ranged from 4.23:16.9 mg/l for lake trout to 8.63:34.5 mg/l for carp in standard reconstituted water at 12° C. Temperature changes had little influence on the effect of the drugs. In very soft water, solutions of the combination are acidic and considerably less toxic than in harder water. The toxicity of the mixture decreases with decreasing pH, especially below pH 6.5. Safety indices (lethal concentration/effective concentration) indicate that the safety margin is greater at shorter exposures.

INTRODUCTION

An anesthetic is an important tool for handling fish during operations such as artificial spawning, marking, weighing, measuring, transporting, and others. MS-222 has been shown to be an effective anesthetic for fish and other coldblooded organisms. The action of MS-222 is characterized by rapid and deep anesthetization, but concentrations that render fish immobile are not tolerated for extended periods (McFarland, 1959; Bové, n.d.; Schoettger and Julin, 1967). Bové (n.d.) identified MS-222 as the methanesulphonate of meta-aminobenzoic acid ethyl ester. The compound is a fine, white crystalline powder which is soluble to 11 percent in water and forms a clear, colorless, acidic, and relatively stable solution.

The anesthetic effect of quinaldine (2-methylquinoline) on fish was first reported by Muench (1958). Schoettger and Julin (1969) further investigated the use of quinaldine as an anesthetic for several species of hatchery-reared fish under a variety of temperature and water quality conditions. The action of quinaldine in fish is characterized by long, safe exposure times, but it does not entirely block reflex movements. Quinaldine occurs in coal tar and is made from aniline, acetaldehyde, and hydrochloric acid. It is a colorless, oily liquid that turns reddish-brown upon exposure to air. Quinaldine is soluble in alcohol, ether, chloroform, and acetone but is insoluble in water (Stecher, 1968).

Schoettger and Steucke (1970) reported mixtures of quinaldine and MS-222 to be synergic for anesthetizing fish. The combination of anesthetics exhibits the rapid sedation and lack of reflex response typical of MS-222 and the long, safe exposure time typical of quinaldine.

Amendments to the Federal Food, Drug, and Cosmetic Act require that chemicals used on fish be registered for their specific uses (Lennon, 1967). The registration of MS-222 has been supported by information on its toxicity or maximum safe exposure to fish (Marking, 1967) and the persistence of residues in fish tissues (Walker and Schoettger, 1967).

Recently, Allen and Sills (1973) synthesized quinaldine sulfate ($QdSO_4$), a salt of quinaldine, which is water soluble and has a less pungent odor than quinaldine. Gilderhus, et al. (1973)

evaluated the efficacy of $QdSO_4$ to 15 species of fish and found it to be as effective, on the basis of active ingredient, as that of quinaldine. The toxicity of $QdSO_4$ to fish under a variety of conditions was determined by Marking and Dawson (1973). Sills, et al. (1973) measured $QdSO_4$ residues in 10 species of fish.

Because $QdSO_4$ is more convenient to use than quinaldine, tests of mixtures of $QdSO_4$ and MS-222 were devised. The purpose of this investigation was to define concentrations of three ratios of the combination which are toxic to various species and sizes of freshwater fish at selected exposure periods in water at three temperatures, four water hardnesses, and four pH's. In addition, the safety must be determined for use pattern concentrations and exposures.

METHODS AND MATERIALS

The $QdSO_4$ (quinaldine sulfate) was synthesized at the Southeastern Fish Control Laboratory, Warm Springs, Ga. The MS-222 (methane sulfonate of meta-aminobenzoic acid ethyl ester) was Finquel(R), marketed by Ayerst Laboratories, Inc.

Static toxicity tests of mixtures of $QdSO_4$ and MS-222 were conducted with 3- to 6-cm fish in glass jars containing 15 l of water according to the methods of Lennon and Walker (1964). Larger fish were exposed to the anesthetics in 45-l polyethylene tanks. The two drugs were tested for toxicity against coho salmon (Oncorhynchus kisutch), rainbow trout (Salmo gairdneri), brown trout (Salmo trutta), brook trout (Salvelinus fontinalis), lake trout (Salvelinus namaycush), carp (Cyprinus carpio), channel catfish (Ictalurus punctatus), bluegill (Lepomis macrochirus), and largemouth bass (Micropterus salmoides). The fish were obtained from fish hatcheries, maintained under a fish culturist's care (Hunn, et al., 1968), acclimated to the test water before the chemical was added, and incinerated after death. Ten fish were exposed to each concentration of the anesthetics, and mortalities were recorded periodically the first day and daily thereafter during the 96-hour tests.

The hardness of the test water was altered by adding selected amounts of reconstituting salts to deionized water, and the pH in certain tests (ranging from 6.5 to 9.5) was adjusted and maintained with chemical buffers (Marking and Dawson, 1973). Temperatures of 7°, 12°, and 17° C were controlled by water baths.

Stock solutions of the anesthetics dissolved in water were added to the bioassays to obtain the desired concentrations. $QdSO_4$ and MS-222 were tested in a ratio of 1:4 against the nine available species, while additional ratios of 1:6 and 1:2 were tested against representative coldwater and warmwater species.

The mortality data were analyzed according to the method of Litchfield and Wilcoxon (1949) to determine LC50's (concentration causing 50 percent mortality), variations, slope functions, and 95-percent confidence intervals.

Fingerling rainbow trout (1.1 g) were exposed to mixtures of $QdSO_4$ and MS-222 (1:2) to determine safety indices (Marking, 1967). A safety index refers to the margin between efficacy and mortality and is expressed by the quotient of a lethal concentration (LC50) and an effective concentration (EC50). The EC50 defines the concentration of drugs which produces total loss of equilibrium (stage 2) in half the organisms (Schoettger and Julin, 1967). The maximum safety index (LC1/EC99) is lower than the safety index and is biased in favor of greater safety.

The toxicity of mixtures of the anesthetics was defined by an additive index[1] developed at the Fish Control Laboratory. The index expresses the toxicity quantitatively with zero indicating strictly additive toxicity. Negative values indicate less than additive toxicity and positive values indicate greater than additive toxicity.

[1] Leif L. Marking and Verdel K. Dawson. A method to assess the toxic or other effects of mixtures of chemicals. (Manuscript)

$\frac{A_m}{A_i} + \frac{B_m}{B_i} = S$, the sum of biological effects

Additive index = $\frac{1}{S}$ -1.0 for $S \leq 1.0$ and

Additive index = [S(-1)] +1.0 for $S \geq 1.0$

where A and B represent concentrations of chemicals. Individual concentrations are designated by i and mixtures of A and B are designated by m.

RESULTS

Effects of $QdSO_4$:MS-222 Combinations on Test Solutions

$QdSO_4$:MS-222 solutions are acidic and influence the pH of bioassay water, especially softer waters. A stock solution containing 30 g of $QdSO_4$ and 60 g of MS-222 in a liter of deionized water has a pH of 1.25. Each chemical decreases the pH of bioassay waters significantly (Marking and Dawson, 1973; Allen and Harman, 1970). The extent of the reduction of pH in waters of various hardnesses by $QdSO_4$:MS-222 solutions in the ratio of 1:2 is given in table 1. Very soft water is poorly buffered, and the pH is lowered more than 40 percent by a $QdSO_4$:MS-222 concentration of 35:70 mg/l. In harder waters the pH is more stable, and in very hard water the pH drops only 12.6 percent at this concentration of the drugs.

The extent to which the pH is decreased appears to be independent of the ratio of the anesthetics. The percentage reduction of the pH was very nearly the same at ratios of 1:2, 1:4, and 1:6 where the total concentration of the two anesthetics was 75 mg/l (table 2).

Species and Sizes of Fish

The toxicity of the mixture of $QdSO_4$ and MS-222 to nine species of fish is presented in table 3. We selected one ratio of the combination (1:4) to scrutinize the effects of the drugs on various species tested. Lake trout are the most sensitive at all exposures to the 1:4 ratio of $QdSO_4$ and MS-222 (LC50 = 7.25:29.0 mg/l at 1 hour and 4.23:16.9 mg/l at 96 hours), and coho salmon are the most resistant (LC50 = 11.3:45.0 mg/l at 1 hour and 6.53:26.1 mg/l at 96 hours) (table 3).

Among the warmwater species tested, largemouth bass are the most sensitive to the $QdSO_4$:MS-222 combination with 1- and 96-hour LC50's of 7.75:31.0 and 5.38:21.5 mg/l, respectively. At a 1-hour exposure to the anesthetics, channel catfish are the most resistant (LC50 = 14.0:56.0 mg/l), but at 96 hours of exposure carp are the most resistant (LC50 = 8.63:34.5 mg/l).

Table 1.--Influence of combinations of $QdSO_4$:MS-222 (1:2) on the pH of test solutions of various hardnesses

Water hardness	Initial pH	Reduction of pH at $QdSO_4$:MS-222 mixtures (mg/l) of					
		15:30		25:50		35:70	
		Final pH	Percentage reduction	Final pH	Percentage reduction	Final pH	Percentage reduction
very soft.....	6.48	4.53	30.1	3.95	39.0	3.74	42.3
soft.....	7.36	6.57	10.7	5.98	18.8	5.60	23.9
hard.....	7.86	7.19	8.52	6.95	11.6	6.75	14.1
very hard.....	8.20	7.57	7.68	7.34	10.5	7.17	12.6

Table 2.--Influence of three ratios of $QdSO_4$:MS-222 (75.0 mg/l total) on the pH of test solutions of various hardnesses

Water hardness	Initial pH	Reduction of pH at $QdSO_4$:MS-222 ratios (mg/l) of					
		25:50		15:60		10.7:64.3	
		Final pH	Percentage reduction	Final pH	Percentage reduction	Final pH	Percentage reduction
very soft....	6.48	3.95	39.0	3.94	39.2	3.96	38.9
soft.....	7.36	5.98	18.8	6.03	18.1	6.02	18.2
hard.....	7.86	6.95	11.6	6.94	11.7	6.96	11.5
very hard....	8.20	7.34	10.5	7.35	10.4	7.38	10.0

Larger sizes of coho salmon and brook trout were exposed to the 1:4 ratio of $QdSO_4$ and MS-222. The 13.2-g coho salmon and 11.6-g brook trout were more resistant than smaller fish of the same species (table 3).

Effects of Temperature and Water Hardness

The toxicity of the drugs to fingerling rainbow trout in soft reconstituted water was not significantly different (P = 0.05) at temperatures of 7°, 12°, and 17° C (table 4). The lack of influence by temperature was evident at all exposure periods and concentration ratios tested.

Water hardness apparently influences the toxicity of mixtures of the two anesthetics. The 96-hour LC50's for rainbow trout in very soft water (12 mg/l of total hardness) were significantly (P = 0.05) greater than in harder waters (table 4). The decreased activity of the combination in very soft water can possibly be attributed to a decrease in pH as indicated in table 2. At a total of 75 mg/l of $QdSO_4$ and MS-222 in ratios of 1:2, 1:4, or 1:6, the pH of the test solution dropped to about 4.0. This is below the pKa value of 5.42 for quinaldine (Knight et al., 1955; Sober, 1968) and is very near the pKa value of 3.5 for MS-222 (Maren et al., 1968). The equilibrium for both chemicals, therefore, is shifted in favor of the ionized form which is relatively unavailable to the fish (Sills and Allen, 1971).

In soft water (44 mg/l of total hardness), the combination is more toxic than in very soft water (12 mg/l of total hardness), and the 96-hour LC50's for the 1:4 ratio against rainbow trout are 5.50:22.0 mg/l and 7.63:30.5 mg/l, respectively. At 96 hours the toxicity of the drugs is insignificantly (P = 0.05) different in soft, hard (170 mg/l of total hardness) and very hard (300 mg/l of total hardness) water (table 4).

Effects of pH

Although solutions were chemically buffered to specific pH's, the acidic nature of the stock solution of the anesthetics caused a reduction in the pH of the bioassay water. Various amounts of 1 N NaOH were added to each test vessel, depending upon the concentration of the anesthetics, to readjust the pH to the original value. A linear regression of the ml of 1 N NaOH required to readjust the pH to its original value versus the total concentration of the combination of anesthetics produced a slope, intercept, and correlation coefficient of 0.08, 0.0, and 0.9995, respectively. The slope indicates that regardless of the initial pH of the solution, 0.08 ml of 1 N NaOH is required for each mg/l of the combination in order to readjust the pH to its original value.

Tests of the combination of $QdSO_4$:MS-222 in the ratio of 1:2 at pH 6.5, 7.5, 8.5, and 9.5 indicate the activity is greater at the higher

Table 3.--Toxicity of combinations of $QdSO_4$ and MS-222 to fish in soft reconstituted water at 12° C

Species	Average weight (g)	Average length (cm)	Ratio of $QdSO_4$: MS-222	LC50 of $QdSO_4$:MS-222 combinations (mg/l) at				
				1 hour	3 hours	6 hours	24 hours	96 hours
Coho salmon....	0.7	4.1	1:6	7.67:46.0	6.98:41.9	6.85:41.1	5.21:31.3	4.93:29.6
Do.....	0.7	4.1	1:4	11.3:45.0	11.0:43.9	10.6:42.4	7.70:30.8	6.53:26.1
Do.....	0.7	4.1	1:2	16.6:33.1	16.6:33.1	16.6:33.1	12.8:25.5	10.3:20.5
Do.....	13.2	10.7	1:4	11.2:44.8	11.2:44.8	11.1:44.2	9.58:38.3	8.55:34.2
Rainbow trout..	0.3	3.1	1:4	10.5:42.0	9.40:37.6	8.05:32.2	6.35:25.4	5.50:22.0
Do.....	0.6	3.8	1:2	16.0:31.9	14.5:29.0	13.5:27.0	9.05:18.1	9.05:18.1
Brown trout....	0.6	3.8	1:6	6.83:41.0	6.50:39.0	6.28:37.7	4.45:26.7	4.45:26.7
Do.....	0.6	3.8	1:4	10.5:42.0	9.05:36.2	8.73:34.9	6.68:26.7	5.73:22.9
Do.....	0.6	3.8	1:2	16.5:33.0	14.9:29.7	13.7:27.3	10.0:20.0	9.15:18.3
Brook trout....	1.2	4.8	1:6	8.58:51.5	8.42:50.5	7.85:47.1	5.95:35.7	4.70:28.2
Do.....	1.2	4.8	1:4	11.0:44.0	10.7:42.7	10.3:41.2	7.23:28.9	6.15:24.6
Do.....	1.2	4.8	1:2	18.9:37.8	18.7:37.3	16.8:33.6	10.9:21.8	9.85:19.7
Do.....	11.6	10.2	1:4	13.2:52.7	11.7:46.6	10.3:41.0	8.70:34.8	7.63:30.5
Lake trout.....	0.5	4.1	1:4	7.25:29.0	6.53:26.1	5.95:23.8	4.25:17.0	4.23:16.9
Carp.....	1.3	4.3	1:6	8.83:53.0	7.22:43.3	6.90:41.4	6.90:41.4	6.68:40.1
Do.....	1.3	4.3	1:4	11.0:44.1	10.2:40.9	9.78:39.1	8.78:35.1	8.63:34.5
Do.....	1.3	4.3	1:2	22.3:44.5	18.1:36.1	16.9:33.8	16.0:32.0	14.7:29.3
Channel catfish	1.8	6.1	1:6	9.25:55.5	9.25:55.5	7.67:46.0	7.23:43.4	5.48:32.9
Do.....	1.8	6.1	1:4	14.0:56.0	11.9:47.7	11.6:46.2	9.25:37.0	7.70:30.8
Do.....	1.8	6.1	1:2	23.4:46.7	20.4:40.7	20.0:40.0	17.2:34.3	11.7:23.3
Bluegill.....	1.5	4.3	1:6	8.50:51.0	5.53:33.2	4.87:29.2	4.87:29.2	4.80:28.8
Do.....	1.5	4.3	1:4	9.25:37.0	7.08:28.3	7.08:28.3	6.93:27.7	6.93:27.7
Do.....	1.5	4.3	1:2	16.1:32.1	13.8:27.5	12.3:24.6	11.0:22.0	11.0:22.0
Largemouth bass	2.8	5.8	1:4	7.75:31.0	6.75:27.0	6.25:25.0	5.50:22.0	5.38:21.5

pH's, especially in longer exposures (table 5). However, the influence of pH in this range is relatively small. The 96-hour LC50's for the combination at pH 6.5 and 9.5 are 8.35:16.7 and 5.90:11.8 mg/l, respectively.

Safety Indices

Safety indices (LC50/EC50) and maximum safety indices (LC1/EC99) were determined for mixtures of $QdSO_4$:MS-222 (1:2) against rainbow trout at 5-, 10-, 15-, and 30-minute exposures (table 6). The safety indices ranged from 4.23 to 2.60 at 5 and 30 minutes, respectively. The safety index values averaged 1.6 times the corresponding maximum safety indices. As the exposure time increased, the safety margin decreased. Therefore, increased safety is achieved by using concentrations that are effective at shorter exposures.

Quantification of Additive Toxicity

Schoettger and Steucke (1970) indicated that the combination of MS-222 and quinaldine was synergic for anesthetizing fish. The extent of the synergism, or the effect of changing the ratio of the two materials, was not fully defined. The additive index was determined for the data to quantitate the extent of synergism at selected ratios of the two chemicals. If the index is greater than zero, synergism or greater than additive effect is indicated.

Table 7 presents the index values for selected species, exposure periods, and ratios of the two anesthetics. In all cases the index values are greater than zero, and the average of all values is 0.26 indicating that the effect of the two chemicals is greater than additive. Statistical analysis failed to show any significant difference in the additive toxicity between any of the ratios tested ($P = 0.05$).

Table 4.--Toxicity of combinations of $QdSO_4$ and MS-222 to fingerling rainbow trout at various temperatures and water hardnesses

Temp. (°C)	Water hardness	Ratio of $QdSO_4$:MS-222	LC50 of $QdSO_4$:MS-222 combinations (mg/l) at						
			0.25 hour	0.5 hour	1 hour	3 hours	6 hours	24 hours	96 hours
7.....	soft	1:2	25.3:50.6	16.5:32.9	15.6:31.2	13.8:27.6	11.0:21.9	9.25:18.5	8.40:16.8
Do.....	soft	1:4	--	--	9.80:39.2	9.10:36.4	8.05:32.2	6.35:25.4	5.50:22.0
12.....	soft	1:2	27.5:55.0	16.2:32.3	16.0:31.9	14.5:29.0	13.5:27.0	9.05:18.1	9.05:18.1
Do.....	soft	1:4	--	--	10.5:42.0	9.40:37.6	8.05:32.2	6.35:25.4	5.50:22.0
17.....	soft	1:2	26.5:52.9	15.4:30.8	14.6:29.2	13.5:27.0	12.5:25.0	8.50:17.0	8.35:16.7
Do.....	soft	1:4	--	--	10.4:41.5	8.80:35.2	7.50:29.0	5.73:22.9	5.40:21.6
12.....	very soft	1:2	--	--	--	34.1:68.2	28.0:56.0	16.8:33.6	--
Do.....	very soft	1:4	--	--	14.8:59.0	12.8:51.3	--	9.75:39.0	7.63:30.5
Do.....	hard	1:2	--	15.6:31.1	14.9:29.8	14.1:28.1	12.1:24.1	8.75:17.5	8.75:17.5
Do.....	hard	1:4	--	--	9.00:36.0	8.38:33.5	--	6.00:24.0	5.73:22.9
Do.....	very hard	1:2	--	15.0:30.0	14.1:28.2	14.1:28.2	12.1:24.1	9.90:19.8	9.05:18.1
Do.....	very hard	1:4	--	--	8.63:34.5	8.18:32.7	--	5.80:23.2	5.55:22.2

Table 5.--Toxicity of combinations of $QdSO_4$ and MS-222 (1:2) to fingerling rainbow trout in soft reconstituted water at 12° C buffered to selected pH's

Time (hours)	LC50 of $QdSO_4$:MS-222 combinations (mg/l) at pH			
	6.5	7.5	8.5	9.5
0.25.....	20.0:40.0	18.0:36.0	24.1:48.1	17.2:34.4
0.50.....	15.3:30.5	15.0:30.0	15.0:30.0	15.5:31.0
1.0.....	13.7:27.4	13.3:26.6	12.5:25.0	10.6:21.1
3.0.....	12.5:25.0	11.5:23.0	9.55:19.1	9.05:18.1
6.0.....	--	--	9.55:19.1	9.05:18.1
24.0.....	9.60:19.2	8.10:16.2	6.20:12.4	6.15:12.3
96.0.....	8.35:16.7	8.10:16.2	6.20:12.4	5.90:11.8

Table 6.--Safety indices for mixtures of $QdSO_4$:MS-222 (1:2) using rainbow trout in soft reconstituted water at 12° C

Exposure (min)	Concentration of anesthetics (mg/l)				Safety indices	
	LC50	EC50	LC1	EC99	LC50/EC50	LC1/EC99
5.....	56.7	13.4	37.5	14.7	4.23	2.55
10.....	42.2	11.5	36.0	13.8	3.67	2.61
15.....	34.5	10.8	25.1	13.1	3.19	1.92
30.....	27.3	10.5	21.8	12.8	2.60	1.70

Table 7.--Toxicity of $QdSO_4$ and MS-222 (LC50's in mg/l) individually and in combination and their additive indices at selected exposure periods

Species	Exposure (hours)	Individually		In combination		Ratio	Additive index
		$QdSO_4$[1]	MS-222[2]	$QdSO_4$	MS-222		
Rainbow trout...	24	37.0	39.0	6.35	25.4	1:4	0.22
Do.....	24	37.0	39.0	9.05	18.1	1:2	0.41
Do.....	96	31.8	38.4	5.50	22.0	1:4	0.34
Do.....	96	31.8	38.4	9.05	18.1	1:2	0.32
Brown trout.....	24	32.7	38.5	4.45	26.7	1:6	0.21
Do.....	24	32.7	38.5	6.68	26.7	1:4	0.11
Do.....	24	32.7	38.5	10.0	20.0	1:2	0.21
Do.....	96	28.3	43.8	4.45	26.7	1:6	0.30
Do.....	96	28.3	43.8	5.73	22.9	1:4	0.38
Do.....	96	28.3	43.8	9.15	18.3	1:2	0.35
Brook trout.....	24	27.2	50.7	5.95	35.7	1:6	0.08
Do.....	24	27.2	50.7	7.23	28.9	1:4	0.20
Do.....	24	27.2	50.7	10.9	21.8	1:2	0.20
Do.....	96	22.2	50.0	4.70	28.2	1:6	0.29
Do.....	96	22.2	50.0	6.15	24.6	1:4	0.30
Do.....	96	22.2	50.0	9.85	19.7	1:2	0.19
Lake trout.....	24	16.3	33.8	4.25	17.0	1:4	0.31
Do.....	96	15.5	32.0	4.23	16.9	1:4	0.25
Bluegill.....	24	36.8	45.7	4.87	29.2	1:6	0.30
Do.....	24	36.8	45.7	6.93	27.7	1:4	0.26
Do.....	24	36.8	45.7	11.0	22.0	1:2	0.28
Do.....	96	32.0	45.7	4.80	28.8	1:6	0.28
Do.....	96	32.0	45.7	6.93	27.7	1:4	0.22
Do.....	96	32.0	45.7	11.0	22.0	1:2	0.21
Largemouth bass.	24	16.0	47.0	5.50	22.0	1:4	0.23

[1] From (Marking and Dawson, 1973)
[2] From (Marking, 1967)

DISCUSSION

The pattern of toxic response of the mixture of $QdSO_4$:MS-222 among various species and sizes of fish is similar to that of each component when tested individually (Marking, 1967; Marking and Dawson, 1973). In both cases lake trout were the most sensitive of the coldwater species tested and largemouth bass were the most sensitive of the warmwater species tested. Also, larger fish of the same species were more resistant than smaller ones.

The effect of temperature, however, does not show a similar pattern when tested individually. Temperature had very little effect on the toxicity of the mixture of the anesthetics to rainbow trout. However, when MS-222 was tested individually the trout were more resistant at lower temperatures. This

was true also of the $QdSO_4$ for longer exposures, but the trend was reversed in 1- to 6-hour exposures.

Tests of the anesthetic mixture at adjusted pH's of 6.5, 7.5, 8.5, and 9.5 indicated the pH had only a slight influence on the toxicity of the anesthetics in this range. This is not surprising considering the pKa value of each of the components is more than one pH unit below the lowest pH tested. According to the Henderson-Hasselbach equation, even at pH 6.5, 92.3 percent of the $QdSO_4$ and 99.9 percent of the MS-222 would be un-ionized. The un-ionized forms of both molecules are lipid-soluble, thereby making both anesthetics potentially available to the fish (Sills and Allen, 1971).

On the other hand, there was a singificant decrease in the pH of poorly buffered solutions. This is because $QdSO_4$ is a water-soluble salt of quinaldine, and MS-222 is a water-soluble salt of m-aminobenzoic acid ethyl ester. Being water-soluble, the salt forms are easier to handle, but the sulfuric acid from $QdSO_4$ and the methane sulfonic acid from MS-222 are strong acids. If the anesthetic mixture were used in soft, unbuffered water, the pH may go below 6.5, and there would be a substantial decrease in both toxicity and efficacy.

The toxicity of the anesthetics is increased when they are combined as indicated by an average additive index of 0.26. The increased toxicity of the combination would be hazardous when the desired effect is sedation and not mortality. However, when the additive index formula is applied to information presented by Berger (1969) on the efficacy of the mixture as an anesthetic, a value of 0.29 is obtained. The index for toxicity and the index for anesthesia are both greater than one, thus indicating that although the mixture is more toxic it also is more effective as an anesthetic. The important advantage in using the mixture is in combining the rapid, deep anesthetization of MS-222 and the long, safe exposure time of $QdSO_4$.

CONCLUSIONS

1. Ninety-six hour LC50's for the 1:4 ratio of $QdSO_4$:MS-222 among nine species of fish ranged from 4.23:16.9 mg/1 for lake trout to 8.63:34.5 mg/1 for carp in soft reconstituted water at 12° C.

2. Larger fish are generally more resistant to the combined anesthetics than smaller fish.

3. The toxic effect of the combination is greater than additive as indicated by an average additive index of 0.26. The additive toxicity of $QdSO_4$:MS-222 ratios of 1:2, 1:4, and 1:6 were insignificantly different ($P = 0.05$).

4. The toxicity of the drugs to fingerling rainbow trout was not influenced by temperature changes from 7° to 17° C.

5. The combination of anesthetics is slightly less toxic in solutions adjusted to pH 6.5 than in solutions adjusted to pH 9.5. The lower pH probably reduces the concentration of the active, un-ionized form of the molecules.

6. The mixture is less toxic in very soft water than in harder water, but the decreased pH in very soft water is considered responsible for the reduced activity.

7. Safety indices indicate that the safety margin is greater at shorter exposures.

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INVESTIGATIONS IN FISH CONTROL

54. The Efficacy of Quinaldine Sulfate: MS-222 Mixtures for the Anesthetization of Freshwater Fish

By Philip A. Gilderhus, Bernard L. Berger,
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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

ABSTRACT.--Combinations of quinaldine sulfate ($QdSO_4$) and MS-222 were tested for their efficacy in anesthetizing 14 species of freshwater fish. The combinations induced rapid and deep anesthesia as does MS-222 and permitted long safe holding times as does $QdSO_4$. The concentrations of the combined anesthetics needed were considerably lower than those needed when MS-222 is used alone. Most salmonids tested required concentrations of 10:20 to 10:40 mg/l ($QdSO_4$:MS-222) for effective anesthetization. Warmwater species generally required higher concentrations of 10:40 to 20:75 mg/l. Large adult fish usually required higher concentrations than smaller fish.

Both compounds lower the pH of the solution, and at pH's approaching 6.0 or below the combinations were much less effective. In soft waters where the pH was lowered to that point, buffering the pH back to 6.5 or higher restored the activity of the anesthetics.

INTRODUCTION

The individual attributes and use patterns of quinaldine and MS-222 as anesthetics for fish have been well documented (Schoettger and Julin, 1967, 1969). Schoettger and Steucke (1970) tested mixtures of quinaldine and MS-222 against rainbow trout[1] and northern pike and found the combinations to possess most of the attributes of both anesthetics. Furthermore, substantially less of each component was necessary when they were used in combination. The combination in concentrations from 5:20 mg/l (quinaldine: MS-222) for rainbow trout to 20:60 mg/l for northern pike, rapidly anesthetized the fish and permitted them to be held safely in the chemical solution for at least 60 minutes.

Most recently Allen and Sills (1973) synthesized quinaldine sulfate ($QdSO_4$), a salt of quinaldine which is more convient to use than quinaldine, because it is water soluble. The efficacy of $QdSO_4$ was found to be essentially the same, on an active ingredient basis, as that of quinaldine (Gilderhus et al. 1973).

Since $QdSO_4$ is a crystalline material, it appeared to be ideal for use in combination with MS-222. The two compounds could be blended together and stored or marketed as a ready-to-use mixture. Using the data of Schoettger and Steucke (1970) as a starting point, our objectives were to determine the effective concentrations and ratios of concentrations of the combined anesthetics for 14 species of fish, and evaluate the influences of water quality and temperature on the efficacy of the anesthetics.

METHODS AND MATERIALS

The quinaldine sulfate ($QdSO_4$) used in these tests was synthesized at the Southeastern Fish Control Laboratory, Warm Springs, Ga. The MS-222 (methane sulfonate of meta-aminobenzoic acid ethyl ester) was Finquel(R), marketed by Ayerst Laboratories, Inc.

[1] The common and scientific names of the fish used in the present study are given in table 1.

The anesthetics for each combination were weighed individually and either introduced directly into the test vessel or mixed into water solution in a flask and then introduced into the test vessel. The same procedures were used for both laboratory and field tests.

Tests were conducted at the Fish Control Laboratories, La Crosse, Wis., and Warm Springs, Ga., depending on the availability of test fish. Fingerling-size fish were exposed to the anesthetics in 15-l glass jars, and 45- and 100-l polyethylene tanks were used for tests with larger fish. The temperature was maintained by placing the test vessel in a circulating water bath equipped with heating or cooling equipment.

The efficacy of the combined anesthetics was evaluated against five species of salmonids and nine species of warmwater fish (table 1). The fish for laboratory tests were obtained from federal or state fish hatcheries except for small coho salmon and rainbow trout which were hatched and reared at the La Crosse laboratory. All fish used in laboratory tests were maintained as described by Hunn et al. (1968). They were acclimated to the test conditions for 16 to 24 hours before the anesthetics were added. Tests were also conducted against the five species of salmonids, northern pike, and walleyes at fish hatcheries during their spawning and marking operations.

The laboratory tests were conducted in well, city, and reconstituted waters at La Crosse and in limed spring water at Warm Springs (table 2). The efficacy of the anesthetics at selected pH's was assessed in reconstituted waters in which the pH was adjusted with a KH_2PO_4-NaOH buffer system (Marking, 1969). In some tests where the anesthetic chemicals lowered the pH of the water below the point where they were effective, the pH was raised by adding $NaHCO_3$. For example, to raise the pH of 45 l of water to 7.0, 1.9 and 11.0 g of $NaHCO_3$ were added to waters of pH 5.3 and 3.8, respectively.

Laboratory tests were conducted at 7°, 12°, and 27° C at La Crosse and at 19° C at Warm Springs. Field tests were conducted at the

Table 1.--Species of fish used in tests of the efficacy of $QdSO_4$:MS-222 mixtures as anesthetics for fish

Common name	Scientific name
Coho salmon	Oncorhynchus kisutch
Rainbow trout	Salmo gairdneri
Brown trout	Salmo trutta
Brook trout	Salvelinus fontinalis
Lake trout	Salvelinus namaycush
Northern pike	Esox lucius
Muskellunge	Esox Masquinongy
Carp	Cyprinus carpio
White amur	Ctenopharyngodon idellus
White sucker	Catostomus commersoni
Black bullhead	Ictalurus melas
Channel catfish ...	Ictalurus punctatus
Bluegill	Lepomis macrochirus
Largemouth bass ...	Micropterus salmoides
Walleye	Stizostedion vitreum

Table 2.--Characteristics of waters used for laboratory tests of $QdSO_4$:MS-222 mixtures as anesthetics for fish

Water type	pH	Total	
		Alkalinity (mg/l)	Hardness (mg/l)
well	7.5-8.0	232-262	238-371
city	7.4-8.2	209-250	289-340
spring[1]	6.8-7.0	([2])	20
Reconstituted			
very soft	6.4-6.8	10-13	10-13
soft.....	7.2-7.6	30-35	40-48
very hard.....	8.0-8.4	225-245	280-320

[1] CaO added to water to prevent osmotic shock in the fish.
[2] Not analyzed.

existing water temperatures of the hatchery water supplies (table 3).

Schoettger and Julin (1967) defined loss of equilibrium, stage 2, as the degree of anesthesia at which locomotion ceases, and opercular rate slows, but there is still some reflex response to pressure on the caudal peduncle. We found that fish anesthetized by mixtures of $QdSO_4$:MS-222 were easily handled when in loss of equilibrium, stage 2. Therefore, the tests were designed to determine the concentrations and ratios of $QdSO_4$:MS-222 which would anesthetize the fish to loss of equilibrium, stage 2, in approximately 4 min or less.

RESULTS

Behavior of the Fish

Fish exposed to the combination of anesthetics generally go through a period of 20 to 30 seconds of normal swimming before becoming sedated. The progression of anesthesia is rapid from sedation to loss of equilibrium, stage 2, at which point it

Table 3.--Characteristics of hatchery water supplies used in tests of $QdSO_4$:MS-222 mixtures as anesthetics for fish

Location	Species tested	Water temp. (°C)	pH	Total	
				Alkalinity (mg/l)	Hardness (mg/l)
Platte River SFH[1] Michigan	Coho salmon	6	7.8	150	168
Manchester NFH[2] Iowa	Rainbow trout	9	7.5	172	215
Manchester NFH Iowa	Brown trout	8	([3])	--	--
Osceola SFH Wisconsin	Brook trout	9	8.1	171	208
Crystal Springs SFH Minnesota	Lake trout (adult)	8	7.5	257	280
Jordan River NFH Michigan	Lake trout (fingerling)	7	7.6	120	120
Lansing SFH Iowa	Northern pike	5	9.2	141	144
Valley City NFH North Dakota	Muskellunge	18	7.9	179	213
Lansing SFH Iowa	Walleye	10	9.2	133	164

[1] State Fish Hatchery.
[2] National Fish Hatchery.
[3] Same water supply as used for rainbow trout. Analysis not done.

either slows or stops. As with quinaldine sulfate alone (Gilderhus et al. 1973), the combination of chemicals rarely induces total loss of reflex. However, the fish are easily handled while in loss of equilibrium, stage 2, and the reflexes which are retained are usually weak and of little consequence to the handler.

Efficacy of the Anesthetics

Combinations of $QdSO_4$:MS-222 proved to be effective anesthetics for all species of fish on which they were tested. Concentrations of 10:20 mg/l ($QdSO_4$:MS-222) were the lowest which were effective for four species of trout in laboratory tests. Coho salmon were slightly more sensitive, requiring a combination of 5:20 mg/l. The salmonids recovered rapidly in fresh water, and they recovered faster in warmer water, requiring up to 4.7 min at 17° C and 20 min at 7° C (table 4).

The larger salmonids exposed to the anesthetics at field stations were somewhat more resistant than the smaller fish exposed in the laboratory, requiring concentrations of 10:20 to 10:40 mg/l (table 5). Brook and lake trout required the highest concentrations under field conditions to subdue them to a handleable condition for artificial spawning. This agrees with Schoettger and Julin (1967) who found brook and lake trout to be more resistant than other trouts to MS-222 alone.

The combination of drugs was less active on most of the species of warmwater fish than on salmonids, and all but small walleyes required higher concentrations. Black bullheads were the most resistant in laboratory tests requiring concentrations of 20:75 mg/l (table 6). Northern pike, carp, and white suckers were the next most resistant requiring 20:50 mg/l to 20:75 mg/l. Small walleyes, the most sensitive fish, were anesthetized by a combination of 5:15 mg/l ($QdSO_4$:MS-222).

Higher concentrations of the combined anesthetics also were required for larger specimens of the warmwater species. Large northern pike required 20:75 mg/l and small northern pike 20:50 mg/l; large channel catfish required 40:60 mg/l and small channel catfish 20:50 mg/l; large walleyes required 10:30 mg/l and small walleyes 5:15 mg/l.

Table 4.--Efficacy of $QdSO_4$:MS-222 combinations as anesthetics for salmonids in laboratory tests

Species	Mean weight (g)	No. of fish	Temp. (°C)	Water type	Concentration (mg/l)	Loss of equilibrium stage 2 (min)	Length of exposure (min)	Recovery in fresh water (min)
Coho salmon...	19	15	7	well	5:20	1.5-2.6	15-60	3.2-5.5
Do.....	16	15	12	soft	10:20	1.4-3.0	15-60	3.1-5.3
Do.....	19	10	12	well	5:20	1.3-1.8	15-30	2.0-3.0
Rainbow trout.	25	15	7	well	10:20	0.9-1.5	15-60	3.0-7.8
Do.....	1.4	15	12	soft	10:40	1.3-4.5	15-60	2.7-10.0
Do.....	1.5	10	12	well	10:20	0.5-0.6	5.5-15	2.0-3.2
Do.....	18	10	12	very hard	10:20	0.9-1.5	15-30	2.0-3.8
Do.....	0.9	15	17	soft [1] pH-7.6	10:20	1.4-3.3	15-60	1.5-3.0
Do.....	0.9	15	17	soft [1] pH-8.5	10:20	1.8-2.9	15-60	2.0-4.5
Do.....	25	15	17	well	10:20	0.8-0.9	15-60	1.0-4.0
Brown trout...	18	10	7	well	10:20	1.1-1.5	15-30	3.0-6.3
Do.....	28	15	12	well	10:20	0.9-1.5	15-60	3.0-7.0
Do.....	16	15	17	well	10:20	1.0-1.3	15-60	2.0-3.8
Brook trout...	27	15	7	well	10:20	0.9-1.4	15-60	3.5-9.4
Do.....	1.1	15	12	soft	10:20	1.0-3.5	5.5-15	1.5-2.6
Do.....	27	15	12	well	10:20	0.9-1.4	15-60	3.0-5.0
Lake trout....	25	10	7	well	10:20	1.6-2.6	15-30	7.0-20.0
Do.....	30	15	12	well	10:20	0.9-1.7	15-60	3.0-11.0
Do.....	25	15	17	well	10:20	1.0-2.5	15-60	2.5-4.7

[1] pH adjusted with buffers.

Table 5.--Efficacy of $QdSO_4$:MS-222 combinations as anesthetics for fish at field stations

Species and location	Mean weight (kg)	No. of fish	Effective concentration (mg/l)	Time (min) to	
				Loss of equilibrium Stage 2	Recovery in fresh water
Coho salmon ..... Platte River SFH[1]	3.5	15	5:10	0.8-2.3	3.0-4.0
Rainbow trout ..... Manchester NFH[2]	3.2	74	10:30	1.2-3.0	3.2-5.0
Brown trout ..... Manchester NFH	2.6	62	5:30	2.0-3.2	1.0-4.5
Brook trout ..... Osceola SFH	1.0	57	10:40	1.2-1.5	2.2-5.5
Lake trout ..... Crystal Springs SFH	2.5	55	10:40	3.0-4.0	4.0-5.0
Lake trout ..... Jordan River NFH	0.01	2,700	10:20	1.2-2.0	4.0-5.5
Northern pike ..... Lansing SFH	1.1	12	20:50	2.5-8.0	8.8-22.0
Muskellunge ..... Valley City NFH	2.3	8	20:50	1.2-1.5	3.0-6.6
Walleye ..... Lansing SFH	0.9	10	10:30	2.5-3.5	6.0-10.0

[1] State Fish Hatchery.
[2] National Fish Hatchery.

Table 6.--Efficacy of $QdSO_4$:MS-222 combinations as anesthetics for warmwater fish in laboratory tests

Species	Mean weight (g)	No. of fish	Temp. (°C)	Water type	Concentration (mg/l)	Loss of equilibrium Stage 2 (min)	Length of exposure (min)	Recovery in fresh water (min)
Northern pike.....	10	15	12	well	10:40	2.4-3.1	15-60	5.5-11.0
Do.....	1791	18	12	well	20:75	3.0-5.0	30	19.0-26.0
Do.....	115	10	17	very soft	20.50	1.7-2.5	5.5-15	3.5-5.9
Do.....	10	15	17	well	10:40	1.8-2.0	15-60	4.5-5.0
Do.....	115	15	22	very soft	20:50	0.9-2.0	5.5-30	3.2-8.5
Carp.....	387	20	12	well	20:50	2.1-3.3	5.5-15	4.5-9.0
Do.....	387	10	27	well	20:50	1.6-2.3	5.5-15	2.5-6.5
White amur.....	227	3	19	spring	20:40	1.5-1.8	30	4.0-5.0
White sucker.....	138	10	12	well	20:50	1.7-2.0	5.5	5.1-7.0
Black bullhead.....	208	10	12	well	20:75	3.3-4.1	5.5-15	10.0-22.0
Do.....	208	10	17	well	20:75	3.5-4.1	15	4.5-16.0
Do.....	129	10	27	well	20:50	2.5-3.1	5.5-15	4.2-6.5
Channel catfish.....	1.8	10	12	well	30:30	1.5-2.3	5.5-15	2.2-14.0
Do.....	1.5	10	17	well	20:50	0.6-1.5	15-30	[1] 1.8-3.5
Do.....	1.8	10	17	very hard	20:50	1.5-2.5	5.5-15	2.0-2.8
Do.....	1316	30	19	spring	40:60	2.0-3.0	30	3.5-7.5
Bluegill.....	77	14	17	well	10:40	1.4-2.1	15-60	5.0-8.2
Do.....	135	49	19	spring	10:40	2.5-3.5	30	1.5-3.0
Do.....	80	15	27	well	10:40	1.1-1.2	5.5-15	1.7-3.0
Largemouth bass.....	12	5	17	well	20:50	1.0-1.1	15	5.0-6.5
Do.....	15	10	17	very hard	20:50	0.8-1.5	5.5-15	2.7-10.0
Do.....	1044	30	19	spring	20:40	1.5-3.1	30	2.5-9.5
Walleye.....	1.1	15	12	well	5:15	1.5-3.3	15-60	5.0-26.0

[1] Some fish killed by 30-minute exposure.

The time required for the fish to recover in fresh water was inversely related to the water temperature with longer times being necessary in colder water. Most fish recovered in less than 10 min, but some needed up to 26 min (table 6).

Water Quality

The efficacy of the combined anesthetics was affected by the chemical characteristics of the water. Both of the compounds are acidic, lowering the pH of the water to which they are added (Allen and Harman, 1970; Marking and Dawson, 1973). We found that in anesthetic solutions of about pH 6 or below, the anesthetics were diminished in efficacy, depending on the concentration. The lowering of the pH was critical only when the anesthetics were placed in soft or very soft, unbuffered water. With rainbow trout, 10:20 mg/l were effective in well water and not effective in very soft water at 12° C. The combination anesthetized northern pike at 10:30 mg/l in well water; whereas 20:75 mg/l were ineffective in soft water at 12° C. Increasing the concentration sometimes compensated for the loss of activity in soft waters, but adding $NaHCO_3$ until the pH of the solution was 6.5 or higher, assured satisfactory activity.

Water Temperature

The water temperature did not decisively or consistently affect the efficacy of the combined anesthetics. The concentrations needed for effective anesthetization of salmonids were the same over a wide range of water temperatures. There was some indication that northern pike and black bullheads might be anesthetized by lower concentrations at higher temperatures but the results were not conclusive. Inconsistent results related to temperature were not surprising. The efficacy of MS-222 apparently is affected by temperature (Schoettger and Julin, 1967), whereas the efficacy of quinaldine is not (Schoettger and Julin, 1969). The recovery time for fish was more consistently related to temperature with recovery being more rapid at higher temperatures.

Repeated Exposure

Repeated anesthetization of the same fish does not appear to affect the sensitivity of the fish to the combined anesthetics. A group of ten 20-cm rainbow trout was anesthetized 11 times in 15 days by a 20:50 mg/l solution and anesthetization and recovery times were unaffected.

Repeated Use of Solutions

The repeated use of solutions of $QdSO_4$:MS-222 was evaluated during the fin clipping of lake trout at Jordan River NFH. We found that 1,800 fish (a total of 25 kg) could be anesthetized in 8 l of a 10:20-mg/l solution before the solution had to be spiked or replaced.

Apparently, raising the concentration slightly from that normally used will help ensure continued effectiveness for a period of several days. Fifty l of a 20:100-mg/l solution were used for 3 days to anesthetize 2.2-3.6 kg northern pike. A total of 135 pike was anesthetized the first day, 125 the second, and 120 the third without noticeable loss of activity of the solution.

DISCUSSION

The combinations of $QdSO_4$ and MS-222 combined the attributes of the individual anesthetics and induced anesthesia more effectively than $QdSO_4$ alone and more safely than MS-222 alone. Fish can be safely held for 1 hour or more in concentrations which effectively anesthetize the respective species. This is in contrast to the 5.5- to 12-minute safe holding times for salmonids in MS-222 given by Schoettger and Julin (1967). An exception was channel catfish which suffered mortalities after 30 min of exposure to 20:50 mg/l, the lowest effective concentration. The long safe holding time afforded by the combination is a distinct advantage because more fish can be anesthetized at one time without danger to the last ones handled. Whereas the combinations do not consistently induce total loss of reflex as does MS-222, they do make fish more handleable than does $QdSO_4$ alone.

The concentrations of the drugs used in combination represent a substantial saving of chemicals over the concentrations necessary when they are used alone. For example, when used alone for salmonids, the concentrations

needed are 80 to 100 mg/l of MS-222 or 25 mg/l of $QdSO_4$. When used in combination, the concentrations necessary to anesthetize salmonids are 10:20 mg/l ($QdSO_4$:MS-222).

The water chemistry appears to be the only factor which consistently influences the efficacy of the combined anesthetics. Apparently, the two chemicals are affected differently, but both have reduced activity in very soft water. Both compounds lower the pH of the water, contributing to the ionization and inactivation of $QdSO_4$ at pH's below 6. MS-222 is less effective in soft waters, apparently because the lack of calcium ions induces osmotic stress in the fish which interferes with the activity of the anesthetic (Schoettger and Julin, 1967).

CONCLUSIONS

1. Combinations of $QdSO_4$ and MS-222 effectively anesthetize a wide variety of fishes.
2. The combinations possess the attributes of both anesthetics--that is, the long safe holding time with $QdSO_4$ and the rapid anesthetization with MS-222.
3. Combining the anesthetics greatly reduces the concentrations over those necessary when they are used alone.
4. Higher concentrations of the combination are generally needed for large adult fish than for small, immature fish.
5. The combination is relatively ineffective if it lowers the pH of the water to 6 or below. This is more prone to occur in soft or unbuffered water.
6. If the combined anesthetics lower the pH of the solution to near 6 or below, the pH should be raised to 6.5 or higher with $NaHCO_3$ or another satisfactory buffer.

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INVESTIGATIONS IN FISH CONTROL

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

United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

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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

ABSTRACT.--Residues of quinaldine and MS-222 in 10 species of fish exposed to mixtures of quinaldine sulfate and MS-222 were determined using gas chromatography and spectrophotometry for quinaldine and colorimetry for MS-222. The residue concentrations of quinaldine and MS-222 decreased rapidly following withdrawal from the anesthetics. The mean concentration of 0-hour residues of quinaldine ranged from 0.15 to 6.92 μg/g depending on concentration, temperature, length of exposure, and species. The mean concentration of 0-hour residues of MS-222 ranged from 1.9 to 27.3 μg/g and decreased to near the background reading of the controls after 24 hours of withdrawal. The fish exposed to the same concentration of the mixture of anesthetics for 15 minutes generally contained higher concentrations of residues than those exposed for 5.5 minutes. Two weight groups of brook trout were exposed to the same concentrations of the mixed anesthetics for the same length of time. The smaller fish contained 1.22 times higher concentration of quinaldine residue and 1.43 times higher concentration of MS-222 residue than the larger fish.

INTRODUCTION

MS-222 (methanesulfonate of meta-aminobenzoic acid ethyl ester) and quinaldine (2-methylquinoline) are effective and widely-used fish anesthetics (Schoettger and Julin, 1967, 1969). The choice of which anesthetic to use depends upon the specific properties that are desired. MS-222 causes rapid immobility, but fish are unable to tolerate long exposures. On the other hand, quinaldine is tolerated for long periods, but does not completely block reflex movement. Schoettger and Steucke (1970) found that mixtures of these drugs offer advantages over the use of each separately. The mixture produced rapid immobility and prolonged toleration at slightly reduced concentrations of each drug.

Allen and Sills (1973) prepared a water-soluble form of quinaldine by forming its sulfate salt. The toxicity of quinaldine sulfate ($QdSO_4$) to fish was determined by Marking and Dawson (1973), and its efficacy as a fish anesthetic was determined by Gilderhus et al. (1973a). The toxicity of the anesthetic mixture (quinaldine sulfate:MS-222) was determined by Dawson and Marking (1973). Gilderhus et al. (1973b) determined the efficacy of the combination anesthetic in the laboratory and under field conditions.

Residue data on four salmonids[1] and channel catfish anesthetized with MS-222 have been reported (Walker and Schoettger, 1967; Schoettger et al., 1967). Allen et al. (1972) determined MS-222 residues in northern pike, muskellunge, and walleye anesthetized with MS-222. Sills et al. (1973) determined residues of quinaldine in five species each of coldwater and warmwater fish following anesthesia with quinaldine sulfate. Sills and Harman (1970) determined quinaldine residues in striped bass (Morone saxatilis) following anesthesia with quinaldine sulfate.

[1]The common and scientific names of fish used in the present study are given in table 1.

Before the mixture of quinaldine sulfate and MS-222 can be registered for general use, more information is needed about the fate of quinaldine and MS-222 in fish tissues. Therefore, this study was undertaken to measure the concentration and persistence of quinaldine and MS-222 residues in five species each of coldwater and warmwater fish following anesthesia with efficacious concentrations of the mixture.

METHODS AND MATERIALS

Ten species of fish (table 1) were exposed quinaldine sulfate and MS-222 (Gilderhus et al., 1973b). Temperatures of treatment ranged from 7° to 19° C, and exposure times ranged from 5.5 to 30 minutes. A wide range of concentrations was necessary, because of the variety of species and temperatures involved.

Withdrawal times began when exposed fish were placed in fresh, flowing water for recovery. At least three fish were collected for residue analysis at 0, 1, 2, 4, either 6 or 8, and 24 hours. Samples of muscle tissue were collected and held frozen until analyzed. Whole fillets were homogenized after thawing to obtain representative samples of edible tissue.

Table 1.--Species of fish analyzed for quinaldine and MS-222 residues following anesthesia with mixtures of quinaldine sulfate and MS-222

Common Name	Scientific Name
Coho salmon	Oncorhynchus kisutch
Brown trout	Salmo trutta
Rainbow trout	Salmo gairdneri
Lake trout	Salvelinus namaycush
Brook trout	Salvelinus fontinalis
Northern pike	Esox lucius
Channel catfish ...	Ictalurus punctatus
Largemouth bass ...	Micropterus salmoides
Bluegill	Lepomis macrochirus
Walleye	Stizostidion vitreum

The samples were analyzed by the colorimetric method of Walker and Schoettger (1967) for MS-222 residue and by the gas chromatographic and U.V. spectrophotometric methods of Allen and Sills (1970a and 1970b) for quinaldine residue. The minimum detectable concentration of the quinaldine methods is 0.01 μg/g and the minimum detectable concentration of the MS-222 method is 0.1 μg/g. Residues of quinaldine less than 0.01 μg/g are reported as zero. The minimum detectable concentration of the MS-222 method is limited also by the background aromatic amines, and all MS-222 results include these.

RESULTS

Coho salmon

Spawning-migrant coho salmon from Lake Michigan were exposed to a mixture of 5 mg of quinaldine sulfate and 10 mg of MS-222 per liter of water at 12° C for 5.5 and 15 minutes (table 2). MS-222 residues ranged from mean concentrations of 1.9 to 3.3 μg/g at the 0-hour interval and decreased to a background level of the controls or slightly above at the 24-hour withdrawal interval. Quinaldine residues ranged from mean concentrations of 0.15 to 0.51 μg/g at the 0-hour interval to zero after 4 to 8 hours of withdrawal. The coho salmon were the largest fish tested.

Brown trout

Brown trout (table 2) were exposed to a mixture of 5 mg of quinaldine sulfate and 30 mg of MS-222 per liter of water at 12° C for 5.5 and 15 minutes. MS-222 residues ranged from mean concentrations of 7.2 to 14.6 μg/g at the 0-hour withdrawal and were within background levels after 8 to 24 hours. Quinaldine residues ranged from mean concentrations of 0.33 to 0.63 μg/g at the 0-hour withdrawal, and were down to zero after 8 hours of withdrawal.

Rainbow trout

Hatchery-reared rainbow trout were tested the most extensively (table 3). Those exposed at 7° C to a mixture of 5 mg of quinaldine sulfate and 30 mg of MS-222 per liter of water for 15 minutes contained a mean concentration

Table 2.--Residues of anesthetics in muscle tissue of coho salmon and brown trout treated with mixtures of quinaldine sulfate ($QdSO_4$) and MS-222 at 12° C for 5.5 and 15 minutes

	Treatment			Mean weight (kg)	Residues (μ/g)[1]			
					Quinaldine		MS-222	
	$QdSO_4$ (mg/l)	MS-222 (mg/l)	Exposure (min)		Mean	Range	Mean	Range
Coho salmon								
Control	0	0	0	2.03	0.00	0.00-0.00	1.1	0.6-1.4
0-hour	5	10	5.5	3.82	0.15	0.09-0.18	3.3	1.6-4.2
1-hour	5	10	5.5	1.53	0.10	0.07-0.14	1.5	1.4-1.6
2-hour	5	10	5.5	3.20	0.02	0.01-0.03	1.5	1.4-1.6
4-hour	5	10	5.5	3.91	0.00	0.00-0.00	0.9	0.6-1.4
8-hour	5	10	5.5	2.71	0.00	0.00-0.00	1.5	1.4-1.6
24-hour	5	10	5.5	1.57	0.00	0.00-0.00	1.1	0.6-2.0
0-hour	5	10	15	3.91	0.51	0.34-0.65	1.9	1.6-2.0
24-hour	5	10	15	4.37	0.00	0.00-0.00	1.8	1.0-2.6
Brown trout								
Control	0	0	0	0.58	0.00	0.00-0.00	0.9	0.6-1.4
0-hour	5	30	5.5	0.44	0.33	0.23-0.40	7.2	5.0-9.0
2-hour	5	30	5.5	0.56	0.01	0.01-0.02	1.0	1.0-1.0
8-hour	5	30	5.5	0.50	0.00	0.00-0.00	0.6	0.6-0.6
0-hour	5	30	15	0.41	0.63	0.55-0.75	14.6	11.4-17.0
2-hour	5	30	15	0.48	0.03	0.02-0.04	1.3	1.0-1.4
4-hour	5	30	15	0.56	0.01	0.01-0.02	0.7	0.6-1.0
8-hour	5	30	15	0.53	0.00	0.00-0.00	1.0	0.6-1.4
24-hour	5	30	15	0.51	0.00	0.00-0.00	0.9	0.6-1.0

[1] Each mean value represents the average of three analyses.

Table 3.--Residues of anesthetics in muscle tissue of rainbow trout anesthetized with mixtures of quinaldine sulfate ($QdSO_4$) and MS-222 at 7°, 12°, and 17° C for 5.5 and 15 minutes

Withdrawal interval	Treatment				Mean weight (g)	Residues (μg/g)[1]			
						Quinaldine		MS-222	
	$QdSO_4$ (mg/l)	MS-222 (mg/l)	Exposure (min)	Temp. (°C)		Mean	Range	Mean	Range
Control.....	0	0	0	7	384	0.00	0.00-0.00	0.7	0.5- 1.0
0-hour.....	5	30	15	7	496	0.29	0.25-0.35	5.2	3.4- 7.6
1-hour.....	5	30	15	7	532	0.24	0.20-0.28	2.4	2.0- 2.8
2-hour.....	5	30	15	7	483	0.13	0.07-0.18	1.9	1.4- 2.8
4-hour.....	5	30	15	7	445	0.02	0.01-0.03	0.8	0.6- 1.0
Control.....	0	0	0	12	135	0.00	0.00-0.00	0.5	0.5- 0.5
0-hour.....	10	40	5.5	12	116	0.72	0.64-0.88	11.9	10.0-13.0
1-hour.....	10	40	5.5	12	148	0.22	0.14-0.29	1.9	1.6- 2.0
2-hour.....	10	40	5.5	12	168	0.03	0.02-0.04	0.6	0.6- 0.6
4-hour.....	10	40	5.5	12	172	0.02	0.01-0.02	0.6	0.5- 0.6
8-hour.....	10	40	5.5	12	155	0.02	0.01-0.03	0.5	0.5- 0.6
24-hour.....	10	40	5.5	12	100	0.00	0.00-0.00	1.0	1.0- 1.0
0-hour.....	10	40	15	12	126	1.44	0.80-2.37	17.0	13.0-24.0
8-hour.....	10	40	15	12	152	0.01	0.01-0.01	0.9	0.6- 1.0
24-hour.....	10	40	15	12	147	0.00	0.00-0.00	1.0	1.0- 1.0
Control.....	0	0	0	17	135	0.00	0.00-0.00	0.7	0.6- 1.0
0-hour.....	5	30	5.5	17	420	0.53	0.48-0.58	6.9	5.2- 8.6
1-hour.....	5	30	5.5	17	412	0.23	0.20-0.26	1.0	0.6- 1.4
2-hour.....	5	30	5.5	17	467	0.12	0.08-0.15	0.8	0.6- 1.0
4-hour.....	5	30	5.5	17	390	0.03	0.02-0.03	0.5	0.5- 0.6
0-hour.....	5	30	15	17	126	1.05	0.72-1.90	16.5	11.6-21.4
1-hour.....	5	30	15	17	148	0.24	0.16-0.32	3.3	3.0- 3.6
2-hour.....	5	30	15	17	145	0.06	0.04-0.10	1.3	1.0- 1.4
4-hour.....	5	30	15	17	118	0.01	0.01-0.02	0.5	0.5- 0.5
8-hour.....	5	30	15	17	152	0.01	0.01-0.01	0.4	0.0- 0.6
24-hour.....	5	30	15	17	155	0.00	0.00-0.00	0.5	0.5- 0.6

[1] Each mean value represents the average of three analyses.

of quinaldine residue of 0.29 μg/g and a mean concentration of MS-222 residue of 5.2 μg/g at 0-hour withdrawal. After 4 hours of withdrawal the MS-222 residue approached the background of the controls and the quinaldine residue had decreased to a mean concentration of 0.02 μg/g.

Rainbow trout exposed at 12° C to a mixture of 10 mg of quinaldine sulfate and 40 mg of MS-222 per liter of water for 5.5 minutes contained a mean concentration of quinaldine residue of 0.72 μg/g and a mean concentration of MS-222 residue of 11.9 μg/g at the 0-hour withdrawal. After 8 hours of withdrawal the MS-222 residue was equal to the background of the controls (0.5 μg/g); however, the 24-hour withdrawal samples showed a residue of 1.0 μg/g of MS-222. The 24-hour withdrawal samples showed no quinaldine residue. Those exposed at 12° C to a mixture of 10 mg of quinaldine sulfate and 40 mg of MS-222 per liter of water for 15 minutes contained a mean concentration of quinaldine residue of 1.44 μg/g and a mean concentration of MS-222 residue of 17.0 μg/g. After 24 hours of withdrawal the MS-222 residue (including background aromatic amines) was still slightly higher (1.0 μg/g) than the background of the controls (0.5 μg/g). The 24-hour withdrawals contained no detectable quinaldine residue.

Rainbow trout exposed at 17° C to a mixture of 5 mg of quinaldine sulfate and 30 mg of MS-222 per liter of water for 5.5 minutes contained a mean concentration of quinaldine residue of 0.53 μg/g and a mean concentration of MS-222 residue of 6.9 μg/g at the 0-hour withdrawal. After 4 hours of withdrawal the MS-222 residue had decreased to within the range of the background in the controls, and the mean concentration of quinaldine residue had decreased to 0.03 μg/g. Rainbow trout exposed at 17° C to a mixture of 5 mg of quinaldine sulfate and 30 mg of MS-222 per liter of water for 15 minutes contained a mean concentration of quinaldine residue of 1.05 μg/g and a mean concentration of MS-222 residue of 16.5 μg/g. After 24 hours of withdrawal the MS-222 residue had decreased to the background of the controls and no quinaldine residue was detected.

Lake trout

Hatchery-reared lake trout were exposed to a mixture of 10 mg of quinaldine sulfate and 40 mg of MS-222 per liter of water at 12° C for 5.5 and 15 minutes (table 4). Those exposed to this combination for 5.5 minutes contained mean concentrations of residues of 0.74 μg/g and 14.1 μg/g of quinaldine and MS-222, respectively. After 24 hours of withdrawal the MS-222 residue had decreased to less than the background of the controls and no quinaldine residue was detected. Those exposed to this combination for 15 minutes contained mean concentrations of residues of 1.26 μg/g and 17.9 μg/g of quinaldine and MS-222, respectively. The 24-hour withdrawal samples showed no residue above the background of the control.

Brook trout

Hatchery-reared brook trout were exposed at 9° C to a mixture of 10 mg of quinaldine sulfate and 40 mg of MS-222 for 5.5 minutes (table 4). Two weight groups were tested. Brook trout weighing approximately 0.3 kg contained mean concentrations of residues of 1.17 μg/g and 9.2 μg/g of quinaldine and MS-222, respectively; those weighing approximately 0.8 kg contained 0.96 μg/g and 6.4 μg/g of quinaldine and MS-222, respectively. Both groups showed no detectable residue above the background of the controls of either anesthetic after the last withdrawal interval.

Northern pike

Spawning adult northern pike from the Mississippi River were exposed to a mixture of 20 mg of quinaldine sulfate and 50 mg of MS-222 per liter of water at 7° C and to a mixture of 20 mg of quinaldine sulfate and 75 mg of MS-222 per liter of water at 12° C for 30 minutes (table 5). The fish treated at 7° C contained mean concentrations of 1.60 μg/g and 9.6 μg/g residues of quinaldine and MS-222, respectively. After 24 hours of withdrawal no residue of quinaldine or MS-222 was detected in this group. Those treated at 12° C contained a mean concentration of quinaldine residue of 1.80 μg/g at the 0-hour withdrawal and no quinaldine residue was detected after 24 hours of

Table 4.--Residues of anesthetics in muscle tissue of lake trout treated with mixtures of quinaldine sulfate ($QdSO_4$) and MS-222 at 12° C for 5.5 and 15 minutes and brook trout treated with mixtures of quinaldine sulfate ($QdSO_4$) and MS-222 at 9° C for 5.5 minutes

Withdrawal interval	Treatment			Mean weight (kg)	Residues (μg/g)[1]			
					Quinaldine		MS-222	
	$QdSO_4$ (mg/l)	MS-222 (mg/l)	Exposure (min)		Mean	Range	Mean	Range
Lake trout								
Control	0	0	0	1.63	0.00	0.00-0.00	0.5	0.5- 0.6
0-hour	10	40	5.5	1.40	0.74	0.55-1.02	14.1	10.0-17.4
1-hour	10	40	5.5	1.47	0.24	0.19-0.27	1.0	1.0- 1.0
4-hour	10	40	5.5	1.27	0.02	0.02-0.03	0.5	0.5- 0.5
8-hour	10	40	5.5	1.78	0.01	0.00-0.02	0.0	0.0- 0.0
24-hour	10	40	5.5	1.35	0.00	0.00-0.00	0.2	0.0- 0.5
0-hour	10	40	15	1.82	1.26	1.05-1.60	17.9	14.8-24.0
8-hour	10	40	15	1.25	0.01	0.00-0.01	0.5	0.5- 0.5
24-hour	10	40	15	1.57	0.00	0.00-0.00	0.2	0.0- 0.5
Brook trout								
Control	0	0	0	0.30	0.00	0.00-0.00	0.6	0.5- 1.0
0-hour	10	40	5.5	0.30	1.17	0.71-1.80	9.2	7.0-13.4
1-hour	10	40	5.5	0.31	0.20	0.17-0.24	1.2	0.6- 2.0
2-hour	10	40	5.5	0.33	0.03	0.02-0.04	0.9	0.5- 1.4
4-hour	10	40	5.5	0.30	0.02	0.02-0.02	0.9	0.5- 1.6
8-hour	10	40	5.5	0.31	0.01	0.01-0.01	0.5	0.5- 0.6
24-hour	10	40	5.5	0.30	0.00	0.00-0.00	0.6	0.5- 1.0
Control	0	0	0	0.80	0.00	0.00-0.01	0.5	0.5- 0.5
0-hour	10	40	5.5	0.88	0.96	0.46-1.31	6.4	3.6-10.4
15-hour	10	40	5.5	0.68	0.00	0.00-0.00	0.5	0.5- 0.5

[1] Each mean value represents the average of three analyses.

Table 5.--Residues of anesthetics in muscle tissue of northern pike treated with mixtures of quinaldine sulfate ($QdSO_4$) and MS-222 for 30 minutes at 7° and 12° C and in muscle tissue of channel catfish treated with the combination anesthetic for 30 minutes at 19° C

Withdrawal interval	Treatment			Mean weight (kg)	Residues ($\mu g/g$)[1]			
					Quinaldine		MS-222	
	$QdSO_4$ (mg/l)	MS-222 (mg/l)	Temp. (°C)		Mean	Range	Mean	Range
Northern pike								
Control.....	0	0	7	1.43	0.00	0.00-0.00	0.6	0.5- 0.6
0-hour.....	20	50	7	1.21	1.60	1.40-1.70	9.6	8.0-12.0
1-hour.....	20	50	7	1.02	0.53	0.48-0.56	2.8	2.8- 2.8
2-hour.....	20	50	7	1.13	0.33	0.22-0.42	1.9	1.6- 2.0
4-hour.....	20	50	7	1.20	0.06	0.05-0.08	1.0	1.0- 1.0
24-hour.....	20	50	7	1.21	0.00	0.00-0.00	0.6	0.6- 0.6
Control.....	0	0	12	1.48	0.00	0.00-0.00	0.6	0.5- 0.6
0-hour.....	20	75	12	1.67	1.80	1.50-1.90	Lost samples	
1-hour.....	20	75	12	2.08	0.34	0.24-0.48	3.0	2.6- 3.6
2-hour.....	20	75	12	1.53	0.07	0.06-0.08	1.4	1.4- 1.4
4-hour.....	20	75	12	1.55	0.04	0.02-0.05	1.0	1.0- 1.0
6-hour.....	20	75	12	1.94	0.02	0.01-0.03	0.5	0.5- 0.6
24-hour.....	20	75	12	1.65	0.00	0.00-0.00	Lost samples	
Channel catfish								
Control.....	0	0	19	1.27	0.00	0.00-0.00	0.3	0.2- 0.6
0-hour.....	40	60	19	1.50	6.92	6.37-7.31	13.7	11.4-15.2
1-hour.....	40	60	19	1.27	3.19	2.96-3.39	4.9	4.2- 6.0
2-hour.....	40	60	19	1.36	1.79	1.52-2.18	3.6	2.8- 5.0
4-hour.....	40	60	19	1.27	0.56	0.44-0.74	0.6	0.2- 1.4
6-hour.....	40	60	19	1.32	0.30	0.10-0.51	0.4	0.1- 1.0
24-hour.....	40	60	19	1.14	0.00	0.00-0.01	0.1	0.0- 0.1

[1] Each mean value represents the average of three analyses.

withdrawal. The 0-hour and 24-hour withdrawal samples of this group for MS-222 analysis were lost; however, the 1-hour withdrawal sample contained a mean MS-222 residue of 3.0 μg/g which is very close to the concentration of MS-222 residue found in the 1-hour withdrawal samples treated at 7° C (2.8 μg/g). The residue of MS-222 in the 6-hour withdrawal samples was within the background of the controls.

Channel catfish

Hatchery-reared channel catfish were exposed to a mixture of 40 mg of quinaldine sulfate and 60 mg of MS-222 per liter of water at 19° C for 30 minutes (table 5). They contained mean concentrations of residues of 6.92 μg/g and 13.7 μg/g of quinaldine and MS-222, respectively. After 24 hours of withdrawal the MS-222 residue was less than the background of the controls and no quinaldine residue was detected.

Largemouth bass

Hatchery-reared largemouth bass were exposed to a mixture of 20 mg of quinaldine sulfate and 40 mg of MS-222 per liter of water at 19° C for 30 minutes (table 6). The mean concentrations of residues of quinaldine and MS-222 at 0-hour were 4.07 μg/g and 15.1 μg/g, respectively. After 24 hours of withdrawal no residues of quinaldine or MS-222 were detected.

Bluegill

Hatchery-reared bluegills were exposed to a mixture of 10 mg of quinaldine sulfate and 40 mg of MS-222 per liter of water at 19° C for 30 minutes (table 6). The mean concentrations of residues of quinaldine and MS-222 at 0-hour were 3.13 μg/g and 27.3 μg/g, respectively. After 24 hours of withdrawal no residues of quinaldine or MS-222 were detected.

Walleye

Spawning adult walleyes from the Mississippi River were exposed to a mixture of 10 mg of quinaldine sulfate and 30 mg of MS-222 per liter of water at 7° C for 30 minutes (table 6). The mean concentrations of residues of quinaldine and MS-222 at 0-hour were 2.20 μg/g and 14.1 μg/g, respectively. After 6 hours of withdrawal from the mixture, the quinaldine residue had decreased to a mean of 0.27 μg/g and the MS-222 residue had decreased to 2.3 μg/g. Only enough fish were available for 6 hours of withdrawal.

DISCUSSION

The decrease in concentration of quinaldine and MS-222 residues during withdrawal of the fish from the mixed anesthetic follow a pattern similar to that of the individual anesthetics (Walker and Schoettger, 1967; Schoettger et al., 1967; Allen et al., 1972; Sills and Harman, 1970; and Sills et al., 1973). After 24 hours of withdrawal residues of both anesthetics decreased to near the background reading of the controls for MS-222 and to less than 0.01 μg/g for quinaldine.

More residues of the two anesthetics were accumulated in smaller brook trout (0.3 kg) than in larger fish (0.8 kg). At the 0-hour withdrawal period 1.22 times more quinaldine residue and 1.43 times more MS-222 were found in the smaller fish than in the larger fish.

MS-222 appears to be taken up by both coldwater and warmwater fish more readily than quinaldine. The mixed anesthetic solutions contained from 1.5 to 6 times higher concentrations of MS-222 than quinaldine sulfate and muscle residues at the 0-hour withdrawal interval contained from 2.0 to 23 times higher concentrations of MS-222 than quinaldine residue.

The warmwater species were exposed to the highest concentrations of the anesthetics at the highest temperature. This group of fish showed slightly higher concentrations of anesthetic residues at the 0-hour withdrawal than the coldwater fish.

The length of exposure influenced the concentration of anesthetic residues as found by the earlier investigators. Fish exposed to the same concentrations of the mixture of anesthetics for 15 minutes contained from 1.2 to 3.4 times the concentration of quinaldine residue and from 1.3 to 2.4 times the MS-222 residue as those exposed for 5.5 minutes, with the exception of coho salmon which showed higher MS-222 residues in the 5.5-minute exposure.

Table 6.--Residues of anesthetics in muscle tissue of largemouth bass and bluegills treated with mixtures of quinaldine sulfate ($QdSO_4$) and MS-222 for 30 minutes at 19° C and walleyes treated with the combination anesthetic for 30 minutes at 7° C

Withdrawal interval	Treatment		Mean weight (kg)	Residues (μg/g)[1]			
				Quinaldine		MS-222	
	$QdSO_4$ (mg/l)	MS-222 (mg/l)		Mean	Range	Mean	Range
Largemouth bass							
Control	0	0	1.09	0.00	0.00-0.00	0.3	0.2- 0.6
0-hour	20	40	1.18	4.07	3.10-4.60	15.1	10.4-19.6
1-hour	20	40	1.04	0.56	0.46-0.62	2.7	1.6- 4.0
2-hour	20	40	1.09	0.49	0.43-0.54	2.6	2.4- 3.0
4-hour	20	40	1.22	0.17	0.14-0.22	0.6	0.1- 1.6
6-hour	20	40	0.91	0.06	0.06-0.07	0.9	0.6- 1.0
24-hour	20	40	1.27	0.00	0.00-0.00	0.2	0.2- 0.2
Bluegill							
Control	0	0	0.12	0.00	0.00-0.00	0.3	0.1- 0.6
0-hour	10	40	0.14	3.13	2.80-3.80	27.3	24.0-32.0
1-hour	10	40	0.15	0.30	0.27-0.34	3.5	2.8- 4.8
2-hour	10	40	0.14	0.14	0.12-0.17	1.5	1.4- 1.6
4-hour	10	40	0.15	0.02	0.02-0.03	0.5	0.2- 0.6
6-hour	10	40	0.17	0.02	0.01-0.04	0.3	0.2- 0.6
24-hour	10	40	0.15	0.00	0.00-0.00	0.1	0.0- 0.1
Walleye							
Control	0	0	0.81	0.00	0.00-0.00	0.7	0.6- 1.0
0-hour	10	30	0.89	2.20	2.00-2.40	14.1	13.6-14.6
1-hour	10	30	1.14	0.86	0.64-1.10	4.6	3.0- 6.2
2-hour	10	30	0.76	0.38	0.17-0.64	4.0	2.8- 5.0
6-hour	10	30	1.02	0.27	0.04-0.53	2.3	1.4- 2.6

[1] Each mean value represents the average of three analyses.

CONCLUSIONS

1. The residues of quinaldine and MS-222 in the species tested varied considerably depending on concentration of the anesthetic, temperature, and length of exposure. As any of these parameters was increased, the residue concentrations at 0-hour withdrawal increased.

2. The residue concentrations of quinaldine decreased to less than 0.01 μg/g and those of MS-222 decreased to near the range of the background of the controls after 24 hours of withdrawal.

3. MS-222 is taken up more readily from the mixed anesthetic solutions than quinaldine.

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