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THE EFFECT OF TRICAINE METHANESULFONATE (M.S. 222) ON THE MOTILITY OF BROOK TROUT SPERM[1]

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During spawn-taking operations in the fall of 1957, Tricaine Methanesulfonate (M.S. 222) was used to anesthetize brood-stock lake trout at the Marquette (Michigan) Fish Hatchery and brook and brown trout at the Grayling Hatchery. An unusually high mortality of the eggs ensued at Marquette, but a good hatch was obtained at Grayling. It was suggested that the difference might have been the result of slight differences in technique used at the two stations, and that the mortality at Marquette might have been caused by the anesthetic. A concentration of 2 grams of M.S. 222 to 7 gallons of water was used at Grayling, and 4 grams to 7 gallons at Marquette; although the anesthetized fish were rinsed in fresh water at both stations, to remove all traces of chemical before spawn-taking, gloves were used for handling the fish at Marquette, but not at Grayling. Water dripped from the gloves into the spawn-collecting pan, presumably carrying with it a small amount of M.S. 222.

On December 11, 1957, tests were made at Marquette to learn what effects various dilutions of M.S. 222 might have on the sperm of brook trout. Although the spawning season was nearly over, some males in the ponds were still ripe. Microscopic examination of the milt revealed a considerable variability in motility of the spermatozoa among different males. Although spermatozoa from some fish were completely immotile, four males produced sufficient quantities of motile cells for testing.

Procedure

A stock solution of M.S. 222 (75.5 p.p.m.) was made by dissolving 2 grams of the chemical in 7 gallons of water. For each test, stock solution was diluted with river water (which supplies the hatchery) to give 400 cc. of solution of a specified concentration of M.S. 222. Each test was made in a 600-cc. glass beaker. Several drops of milt were expressed from a fish into the solution and the contents stirred immediately; the time was checked on an electric clock with a sweep second hand. To check motility, a drop of the mixture of milt and solution was placed on a glass slide, covered with a cover glass and examined under low power of a compound microscope. The first check was made 10 seconds after the milt was put in the solution, the second check 20 seconds later, and subsequent checks at 30-second intervals, until no further motility was observed. Fresh samples were taken from the mixture for each examination. Spermatozoa from all fish were checked in river water to be sure they were motile. Checks with river water were made three times during the 11 tests with fish no. 4, because it was handled for a much longer time than the other specimens.

Results

Of five males which produced motile spermatozoa, two were used for tests of sperm viability in fresh water only, and three were used for tests in fresh water (control) and in dilutions of M.S. 222 and urethane (Table 1). In fresh water,

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[1] A brief summary of this study was published by Sandoz Pharmaceuticals, Sandoz, Inc., Hanover, New Jersey, in an undated Technical Bulletin, "M.S. 222-Sandoz. The anesthetic of choice in work with cold-blooded animals."

Table 1.--Duration of motility of brook trout spermatozoa in fresh water, urethane, and M.S. 222, December 11, 1957

[+ = sperm motile, 0 = sperm immotile]

Test number	Male number	Solution[1]	Time (seconds) 10	30	60	90	120	150	180	210	240
1	1	FW	+	+	+	+	+	0			
2	2	FW	+	+	+	+	+	0			
3	3	FW	+	+	+	+	+	+	+	+	0
4	3	MS (75.5)	0	0							
5	3	UR (2,995)	0	0							
6	4	MS (75.5)	0	0							
7	4	UR (2,995)	0	0							
8	4	FW	+	+	+	+	+	0			
9	4	MS (37.7)	0	0							
10	4	MS (18.9)	0	0							
11	4	FW	+	+	+	+	+	+	0		
12	4	MS (9.4)	+	0	0						
13	4	MS (9.4)	+	+	0	0					
14	4	MS (4.7)	0	0							
15	4	MS (4.7)	0	0							
16	4	FW	+	+	+	+	0				
17	5	FW	+	+	+	+	+	0			
18	5	MS (18.9)	0	0	0						
19	5	MS (9.4)	+	+	+	+	+	0			
20	5	MS (4.7)	+	+	+	+	+	0			
21	5	MS (1.9)	+	+	+	+	0				

[1] FW = fresh water; MS = M.S. 222; UR = urethane. Concentration in p.p.m. is given in parentheses.

spermatozoa remained motile for 90 to 210 seconds. In the presence of M.S. 222, spermatozoa remained motile for less than 10 seconds at concentrations of 18.9, 37.7, and 75.5 p.p.m., and for more than 10 seconds in 4 out of 7 tests at concentrations of less than 18.9 p.p.m. The present tests were not adequate to establish tolerance limits more precisely. In urethane at a concentration of 2,995 p.p.m., spermatozoa remained motile for less than 10 seconds.

Conclusion

It is possible from these limited tests to conclude that M.S. 222 rendered spermatozoa immotile at dilutions of 18.9 p.p.m., or stronger. This dilution is one-fourth the strength of the solution generally recommended for anesthetization of fish (2 grams in 7 gallons of water) and one-eighth the strength used in the unsuccessful operation at Marquette. Therefore, in spawn-taking operations it is necessary to take precautions to prevent anesthetizing solutions of M.S. 222 from contacting the reproductive products. (The same applies to urethane, at a concentration of 2 ounces per 5 gallons of water.)

Russell Robertson and Charles Shirtz of the State Fish Hatchery, Marquette, Michigan, assisted with this study.

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