U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Wildlife Services

INVESTIGATIONS IN FISH CONTROL

16. Annotated Bibliography on MS-222

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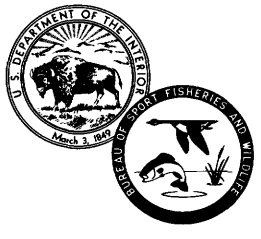

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Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
Resource Publication 22 • January 1967 • Washington, D.C.

ANNOTATED BIBLIOGRAPHY ON MS-222

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Abstract.--This bibliography contains 86 selected references on uses of MS-222 on cold-blooded animals including fish and amphibians. Most of the references are annotated.

The Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., initiated studies in 1964 on the toxicity, efficacy, and residues of MS-222 in fish. The data were required by the U.S. Food and Drug Administration in order to clear the drug for continued use. In connection with the studies, a substantial bibliography on uses of MS-222 was compiled. A considerable number of the more useful references were annotated.

Some recognized references on MS-222 were not available conveniently for review and they have been included by title only.

BIBLIOGRAPHY

Allison, Leonard N.
1961. The effect of tricaine methanesulfonate (M.S. 222) on motility of brook trout sperm. Progressive Fish-Culturist, vol. 23, no. 1, p. 46-48.

The spermatozoa of brook trout remained motile less than 10 seconds in a concentration of 18.9 p.p.m. of MS-222. Allison recommended that the drug should not contact reproductive products during spawn-taking.

Bailey, Merryll M.
1965. Lake trout fin-clipping rates at two national fish hatcheries. Progressive Fish-Culturist, vol. 27, no. 3, p. 169-170.

MS-222 was used as an anesthetic during the fin-clipping of more than 3.5 million lake trout. The fish ranged from 4.1 to 7.9 inches long and were young-of-the-year to 3-year-olds. One fin each was removed from 2 million fish, and 2 fins each were clipped on 1.5 million. Postmarking mortality usually was 0.1 to 0.2 percent; it increased with temperatures over 65° F. Cooling the solutions and reducing the concentration of MS-222 lowered mortality.

Ball, J. N., and P. N. Cowen.
1959. Urethane as a carcinogen and as an anaesthetic for fishes. Nature, vol. 184, p. 370.

Urethane is carcinogenic in the lung and other tissues of mice and rats. It does not produce tumors in rabbits, chickens, and guinea-pigs, but carcinogencity may vary with species (and strain). It has a leucopenic effect on humans and can be absorbed from the skin.

MS-222 has not been reported to be carcinogenic. Its effectiveness on fish and amphibians varies with species, size of the animal, and temperature. Suitable concentrations are determined empirically for every species and situation.

Baudin, Louis.
1932a. Action de la tricaine sur la consommation d'oxygene de Carassius auratus. Comptes rendus des Seances de la Société de biologie, vol. 109, p. 731-733.

A 1:1,000 solution of MS-222 anesthetized goldfish within 1 to 2 minutes. Observations

on the circulation of blood in the fins revealed a slower than normal rate with prolonged exposure to the anesthetic. A concentration of 1:20,000 produced a level of anesthesia in 30 to 60 minutes at 16.5° C. which could be maintained for 24 hours without apparent damage. The rate of oxygen consumption was depressed to 50 percent of normal after 1 hour and to 25 percent after 11 hours of exposure. The respiratory rate returned to normal within 2 hours after the fish were placed in fresh water. Above 16° C., narcosis was incomplete and the reduction of oxygen consumption was less. Below this temperature, a longer period was required for anesthesia and, for a fixed period, the rate of oxygen consumption was higher than at 16.5° C.

1932b. Action de la tricaine sur le quotient resperatoire de Carassius auratus. Ibid., p. 1081-1083.

1932c. Perte de la sensibilité a la dépression chez les poissons anesthésiés a la tricaine. Ibid., vol. 110, p. 151-153.

Oxygen consumption by goldfish was increased at lower atmospheric pressures. The rates for fish which were anesthetized with 1:20,000 of MS-222 were relatively low under conditions of both normal and reduced pressure. The sensitivity of the fish to reduced pressure was not completely suppressed by concentrations of 1:30,000 and 1:40,000.

1932d. Respiration du poisson (Carassius auratus) anesthésié à la tricaïne et soumes à une élévation brusque de température. Ibid., p. 235-237.

1934. Action de la tricaïne sur le sang des poissons. Ibid., vol. 115, p. 510-512.

A 1-percent solution of MS-222 anesthetized several species of fish, including goldfish and perch, within 1 minute. There were no effects on the erythrocytes, erythrocyte count, or oxygen capacity of the blood. The oxygen saturation of the blood was near zero, and the carbon dioxide level was slightly in excess. Lower concentrations of the drug and longer exposures induced exaggerated respiratory movement which increased the oxygen saturation and lowered the carbon dioxide level. The author concluded that MS-222 may excite respiratory centers of the brain, or interfere with exchanges of gases, thereby causing anoxia and hyperventilation. With complete narcosis, the blood showed signs of asphyxia and there was a slowing of the circulation which contributed to reductions in erythocyte numbers and oxygen capacity of the blood.

Bell, Gordon R.

1964. A guide to the properties, characteristics, and uses of some general anaesthetics for fish. Fisheries Research Board of Canada, Bulletin 148, 4 p.

Properties of eleven chemicals used as fish anesthetics are summarized. The chemicals are carbon dioxide, chloral hydrate, chloretone, ether, methyl pentynol, MS-222, phenoxyethanol, quinaldine, sodium amytal, tribromoethanol, and tertiary amyl alcohol. The common and chemical names of the chemicals, manufacturers, costs, solubilities, stabilities, hazards, toxicities, emergency treatments, approximate dosages, precautions, behavioral effects, uses, and modes of action are included. Concentrations of MS-222 from 1:9,000 to 1:4,500 were recommended for brief exposures. The generally useful range is between 1:25,000 to 1:12,500.

Black, Edgar C., and Anne R. Connor.

1964. Effects of MS 222 on glycogen and lactate levels in rainbow trout (Salmo gairdneri). Journal of the Fisheries Research Board of Canada, vol. 21, no. 6, p. 1539-1542.

A concentration of 0.5 g. of MS-222 per gal. anesthetized trout within 60 seconds. The fish showed signs of initial stimulation. The dosage might have been fatal with prolonged exposure. The hemoglobin, blood and muscle lactate, and muscle glycogen levels were similar in anesthetized and control fish.

Blahm, T. H.

1961. Effect of tricaine methanesulfonate on oxygen consumption of juvenile sockeye salmon. Transactions of the American Fisheries Society, vol. 90, no. 2, p. 226-227.

MS-222 uniformly reduced the oxygen consumption of both large (160-180 mm. fork

length) and small (60-80 mm. fork length) sockeye salmon. The fish were anesthetized by 1:20,000 in a constant flow respirometer.

Bové, Frank J.
1962. MS-222 Sandoz--the anaesthetic of choice for fish and other cold-blooded organisms. Sandoz News, no. 3, 12 p.

The author summarizes the history of MS-222 from its use as a local anesthetic in human medicine to its current status as a general anesthetic for fish, amphibians, and other cold-blooded forms. His personal communications with various investigators reveal that concentrations ranging from 1:3,785 to 1:17,500 have been used in measuring and weighing, marking, and spawning of various salmonids. A concentration of 1:12,000 seemed to be most popular. Solutions containing approximately 1:3,000 of MS-222 were effective on tropical fish, goldfish, bluegills, largemouth bass, and bullheads.

Effective dosages for amphibians ranged from 1:250 to 1:20,000, but 1:1,000 to 1:3,000 were preferred. Specimens with gills and those undergoing metamorphosis were more sensitive to the anesthetic.

Butler, Robert L.
1957. The development of a vinyl plastic subcutaneous tag for trout. California Fish and Game, vol. 43, no. 3, p. 201-212.

MS-222 at 0.5 g./gal. prepared fish for the operation within 30 seconds at 50° F. The fish were exposed no longer than 3 minutes. As temperature increases, it is necessary to reduce the exposure time or the concentration. The drug had no effect on feeding by the fish after they recovered from anesthetization.

Campbell, G. D., and D. H. Davies.
1963. Effect of ethyl m-aminobenzoate (MS-222) on the elasmobranch electrocardiograph. Nature, vol. 198, no. 4877, p. 302.

MS-222 caused coincidental decreases in the pulse and respiratory rates of stingrays indicating a neurological relationship between the cardiac and respiratory centers in the brain. The drug did not affect the ECG complex.

Christensen, K.
1931. Effect of castration on the secondary sex characters of males and females of Rana pipiens. Anatomical Record, vol. 48, p. 241.

Collins, James L., and Andrew H. Hulsey.
1963. Hauling mortality of threadfin shad reduced with M.S. 222 and salt. Progressive Fish-Culturist, vol. 25, no. 2, p. 105-106.

A combination of 0.5-percent salt and MS-222 equivalent to 1 g./12 gal. facilitated hauling of more than 200,000 fish with a survival of 95 percent.

Copenhaver, W. M.
1939. Initiation of beat and intrinsic contraction rates in the different parts of the Amblystoma heart. Experimental Zoology, vol. 80, p. 139.

Crawford, Bruce, and Andrew Hulsey.
1963. Effects of M.S. 222 on the spawning of channel catfish. Progressive Fish-Culturist, vol. 25, no. 4, p. 214.

The anesthetization of adult channel catfish with MS-222 at a rate of 4 g./8 gal. did not affect the success of spawning nor the viability of fry. The fish were narcotized for sexing and placement in spawning pens.

Dollar, Alexander M.
1963. Air transportation of living rainbow trout. Progressive Fish-Culturist, vol. 25, no. 3, p. 167-168.

The fish were placed in lake water containing MS-222 at a concentration of 1:100,000. The water was cooled gradually over a 3-hour period at 33° F. The fish and water were transferred to a plastic bag and packed in ice. Oxygen was added to the bags. The fish survived in the sealed containers for 24 hours.

Eisler, Ronald, and Tadeusz Backiel.
1960. Narcotization of chinook salmon fingerlings with tricaine methanesulfonate (M.S. 222). Transactions of the American Fisheries Society, vol. 89, no. 2, p. 164-167.

A concentration of 1:33,000 effectively anesthetized this species within 5 minutes.

The rate of narcotization was dependent on concentration. Recovery time increased with exposures up to an hour, but declined with longer exposure. The drug was equally active in fresh and salt water. The authors reviewed concentrations of MS-222 which have been used to anesthetize various species of fish.

Ellis, Robert J.

1964. The effect of confinement on blood lactate levels in chinook and coho salmon. Research Briefs, Fish Commission of Oregon, vol. 10, no. 1, p. 28-34

The lactic acid levels in the blood of troll-caught salmon declined more rapidly in those individuals which were held in solutions containing MS-222 at a concentration of 1:150,000.

Ewing, Ann.

1965. Current U.S. patents. A method for marking fish by which scales are transplanted painlessly from one part of the body to another has been patented. Science News Letter, vol. 87, no. 15, p. 228.

Dr. Louis Levy and Miss Carol A. De Fusco (1965) have patented a method of marking fish by replacing scales of one color from one part of the body with those of contrasting color from a different area. The fish are anesthetized during the operation with 50 to 100 p.p.m. of MS-222.

The technique was successful on goldfish, carp, blue acara, blackspot barb, and guppies, and has been used to measure the effects of drugs on the rejection time of scales from different fish.

Friddle, S. B., and S. F. Snieszko.

1950. Effect of tricaine methanesulfonate on the determination of sulfonamides. Science, vol. 112, no. 2902, p. 181-182.

Concentrations of 2 to 4 mg.%, as sulfamerazine, were detected in the tissues of trout which should not have contained the sulfa drug. The fish had been anesthetized for 1 minute in a 1:5,000 solution of MS-222. This anesthetic, or others with similar molecular structures, should not be used whenever they may interfere with the colorimetric test for sulfonamides.

Fromm, Paul O.

1958. A method for measuring the oxygen consumption of fish. Progressive Fish-Culturist, vol. 20, no. 3, p. 137-139.

MS-222 is used to immobilize fish for length and weight measurements before they are placed in a respirometer. A concentration of 0.03 percent produces anesthesia within 30 to 45 seconds. The drug apparently has no lasting effect on the general metabolic rate.

Gebhards, Stacy V.

1965. Transport of juvenile trout in sealed containers. Progressive Fish-Culturist, vol. 27, no. 1, p. 31-36.

The use of sedating levels of MS-222 did not increase the loading density or survival of rainbow trout in sealed containers. Greater survival was associated with the starvation period prior to loading.

Gilbert, P. W., and F. G. Wood.

1957. Methods of anaesthetizing large sharks and rays safely and rapidly. Science, vol. 126, p. 212.

A 1:1,000 solution of MS-222 is sprayed into mouth, spiracles, or gill exits by means of a hand sprayer, such as a water pistol. Sharks as large as 400 pounds are anesthetized in a minute or less and may be handled, out of water, for 5 to 30 minutes. The volume of anesthetic solution required to anesthetize fish varies with the size of the individuals.

Glücksohn, Salome.

1932. Äussere Entwicklung der Extremitäten und Stadieneinteilung der Larvenperiode von Triton taeniatus Leyd. und Triton cristatus Laur. W. Roux' Archiv fur Entwicklungsmechanik der Organismen, vol. 125, p. 341-405.

Metamorphosing salamanders were anesthetized with 1:3,000 of MS-222 for 30 minutes a day. The growth of treated specimens was somewhat less than controls, but the former were proportioned normally.

Goodrich, H. B., and R. Nichols.

1931. The development and regeneration of color pattern in Brachydanio rerio. Anatomical Record, vol. 51, p. 513.

Gossington, Robert.

1957. An aid to fish handling--tricaine. Aquarium Journal, vol. 28, no. 9, p. 318-321.

Fish could be anesthetized safely with MS-222 in concentrations of 0.24 to 0.32 g./gal. Larger specimens required somewhat higher concentrations. Live-bearers were generally more resistant than egg-layers. The drug reduced the chances of injury to fish from thrashing during shipment. Under anesthesia, Siamese fighting fish could be shipped together in a single container.

Hadian, Z., and M. S. Dunn.

1938. Localisation in the occulomotor nuclei of the goldfish. Journal of Comparative Neurology, vol. 68, p. 191.

Hublou, Wallace F.

1957. A method of using an anesthetic in marking fins. Progressive Fish-Culturist, vol. 19, no. 1, p. 40-43.

MS-222 was used in a recirculating system to fin-clip fingerling salmon and steelhead trout. The marking rate was improved by 49.5 percent with the use of an anesthetic. Advantages of the system included better marks, lower rate of injury, reduction of worker fatigue, and saving in time and money.

Johnson, Harlan E., and J. M. Shelton.

1958. Marking chinook salmon fry. Progressive Fish-Culturist, vol. 20, no. 4, p. 183-185.

Groups of 10 to 20 individuals were caught in a small net and placed in a 1:7,500 solution of MS-222. Fin-clipping began when all the fish were on their sides and after the net had been placed in fresh water. The last fish was marked just before it recovered. Approximately 487,000 fry were marked at a cost of $50.

Karczmar, Alexander G., and Theodore Koppanyi.

1948. Action of central nervous system depressants at different growth periods of salamander (Amblystoma punctatum) larvae. Federation Proceedings, vol. 7, p. 231-232.

Larvae of different ages were immersed in a 1:7,500 solution of MS-222, and the times for anesthesia were recorded. Paraldehyde, ethyl alcohol, sodium barbital, nembutal, chloral hydrate, and chloretone were also tested. Anesthesia with MS-222 was more rapid in older and larger individuals.

Klontz, George W.

1964. Anesthesia of fishes. From: Proceedings of the Symposium on Experimental Animal Anesthesiology, Brooks Air Force Base, December 14-16, 13 p.

The efficacy and characteristics of 14 methods which are used to anesthetize fish were discussed.

Concentrations of 25 to 35 p.p.m. of MS-222 are recommended for transporting fish; 50 to 100 p.p.m. are used to induce deep anesthesia. In general, induction time requires 1 to 3 minutes of exposure and the fish recover, in fresh water, within 3 to 15 minutes. Fish which are repeatedly exposed to MS-222 showed a slight increase in tolerance which is corrected by raising the concentration slightly. The drug appears to be toxic to those fish which are treated in salt water and in direct sunlight.

Knight, Alexis E.

1964. Intracellular hemoglobin crystallization in two centrarchids, the largemouth bass and the bluegill. Progressive Fish-Culturist, vol. 26, no. 3, p. 115-117.

A 1:5,000 solution of MS-222 was used to narcotize fish during the collection of blood samples.

Koppanyi, Theodore, and Alexander G. Karczmar.

1948. Comparison of anesthetic action of acetanilid, tricaine (MS-222) and aliphatic depressants. Federation Proceedings, vol. 17, p. 234.

The action of acetanilid on salamander larvae was independent of larval stage. Subanesthetic dosages of the drug acted additively with subanesthetic levels of MS-222, nembutal, and chloretone. The anesthetic effects of chloretone, MS-222, alcohol, paraldehyde, and acetanilid were reversed rapidly.

Lemarque, Pierre.
1964. Anesthesie et transport. Bull. Int. Cons. Sup. Pêche, vol. 55, p. 5-9.

MS-222 may be used in concentrations of 1:10,000 to 1:50,000 to anesthetize fish before they are placed in plastic bags at a loading level of 1 kilogram of fish per 1 to 2 liters of water. The bags were filled with oxygen. Dosages of 1:100,000 were recommended for the tranquilization of fish in transportation tanks.

Larsen, Howard N.
1964. Comparison of various methods of hemoglobin determination of catfish blood. Progressive Fish-Culturist, vol. 26, no. 1, p. 11-15.

The fish were anesthetized in a 1:5,000 solution of MS-222 to facilitate the collection of blood samples.

Levy, Louis Encino, and Carol A. DeFusco.
1965. Identification of scaly teleosts. U.S. Patent Office, Patent No. 3,174,458. 3 p.

Lumb, William V.
1963. Small animal anesthesia. Chapter: Anesthesia of laboratory and zoo animals, p. 269-310. Lea and Febiger, Philadelphia. 420 p.

MS-222 is used to immobilize fish and other cold-blooded animals by completely bathing small subjects, by gill spraying in large fish, or by injection in large animals. Concentrations of 0.5 to 1.0 grams of drug per gallon are used for most teleosts and the temperature is maintained at 40° to 60° F. Repeated use of anesthetic solutions reduces their efficacy. Longer anesthesia or sedation can be maintained with lower concentrations. The drug can also be used in treatment of fungus infections and other localized diseases on pet or ornamental fish.

The uses of ether, sodium amytal, carbon dioxide, urethane, and cresol in anesthetizing fish are discussed.

Marking, Leif L.
1966. Investigations in Fish Control. 12. Toxicity of MS-222 to selected fishes. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 18.

The 24-hour LC_{50} concentrations of MS-222 for various species of fish, at 12° C., were found to be: rainbow trout, 39.0 to 52.0 p.p.m.; brown trout, 38.5 to 45.6 p.p.m.; brook trout, 50.7 to 52.2 p.p.m.; lake trout, 33.8 to 39.8 p.p.m.; northern pike, 56.0 p.p.m.; bluegill, 45.7 to 46.9 p.p.m.; largemouth bass, 42.0 to 61.5 p.p.m.; and walleye, 49.0 p.p.m. The results indicated that exposures of MS-222 for 24 to 96 hours had no significant effect on the toxicity of the chemical.

In general, MS-222 was more toxic to smaller individuals, and at higher temperatures. Water hardness had little effect on toxicity.

The safety index of MS-222 for rainbow trout was determined by comparisons of the LC_{50} and EC_{50} concentrations after 15, 30, and 60 minutes of exposure in relatively soft and hard water. The indexes ranged from 1.7 to 2.0 and decreased slightly with exposure time. A comparison of the LC_1 and EC_{99} for hard water gave an index of about 1.3 and 1.0 to 1.1 in soft water.

Maintenance of anesthesia in fish for 96 hours was not harmful. After the fish had been placed in fresh water and recovered, they fed as well as controls.

Martin, N. V., and D. C. Scott.
1959. Use of tricaine methanesulfonate (M.S. 222) in the transport of live fish without water. Progressive Fish-Culturist, vol. 21, no. 4, p. 183-184.

Hybrid trout were anesthetized in 60 p.p.m. of MS-222 and packed in layers of chipped ice and sphagnum moss. The fish were maintained under these conditions for 4 to 4.5 hours with little mortality.

McFarland, William N.
1959. A study of the effects of anesthetics on the behavior and physiology of fishes. Publications of the Institute of Marine Science, University of Texas, vol. 6, p. 23-55.

The anesthetic effects of 21 chemicals, including MS-222, were tested against Fundulus parvipinnis, Gambusia affinis, Paralabrax clathratus, and Girella nigricans. The behavioral changes induced in fish by anesthetics were classified into the following levels of anesthesia: sedation, loss of

equilibrium, loss of reflex reactivity and medullary collapse. Anesthesia in fish was compared to that in humans and was found to be a similar process involving sequential suppression of higher to lower central nervous centers.

The narcotic potencies of the various compounds increased with their molecular weights. MS-222 was rated as highly potent. The ratio of the dosages necessary to induce sedation and medullary collapse during a 12-hour period was 7.1.

Anesthesia with MS-222 was more rapid at 27° than at 12° C., but anesthesia did not progress as deeply at the lower temperature. Metabolic studies indicated that MS-222 was depleted, with time, at a greater rate than other anesthetics.

McFarland, William N.
1960. The use of anesthetics for the handling and the transport of fishes. California Fish and Game, vol. 46, no. 4, p. 407-431.

MS-222, tertiary amyl alcohol, and methylparafynol were suggested as beneficial for the induction of deep anesthesia because they act quickly and recovery is rapid. Recovery from anesthesia was complete provided respiratory movements had not ceased for more than a few minutes. MS-222 at 0.03 g./gal. induced loss of reflex in Fundulus parvipinnis within 1 hour. Higher concentrations were recommended for more rapid anesthesia; however, fish must be removed from the anesthetic after the desired stage of anesthesia has been induced.

MS-222 was not recommended for transporting fish. The drug failed to maintain a lowered rate of metabolism at higher temperatures.

It is advisable to pretreat fish in an anesthetic before transporting to reduce metabolic rates which may be stimulated due to handling.

McGovern, Beulah H., and Roberts Rugh.
1944. Efficacy of m-amino ethyl benzoate as an anesthetic for amphibian embryos. Proceedings of the Society for Experimental Biology and Medicine, vol. 57, p. 127-130.

Dosages of 1:3,000 did not affect the motility or fertility of frog spermatozoa. The eggs which were fertilized in this solution developed normally when the exposure did not exceed 1 hour. Longer exposures produced decreasing numbers of abnormal embryos as development progressed through gastrulation and neurulation. The older embryos withstood anesthesia for 24 hours; however, mortality of the embryos undergoing transition from external to internal gill respiration increased with immersions in MS-222 longer than 2 hours. The drug inhibited muscular action, but not ciliary activity. MS-222 was considered to be nontoxic to frog embryos within the exposure times adequate for surgical operations.

Meehan, William R., and L. Revet.
1962. The effect of tricaine methanesulfonate (M.S. 222) and/or chilled water on oxygen consumption of sockeye salmon fry. Progressive Fish-Culturist, vol. 24, no. 4, p. 185-187.

The most favorable conditions for survival of fish during transportation appeared to be uncrowded numbers of fish in water colder than that from which they were removed. The fish also survived well when uncrowded in their normal environmental water to which was added 0.1 g. of MS-222 per 4,000 ml. Unsatisfactory results were obtained with crowding, or when the fish were placed in solutions of MS-222 which were colder than their environmental water.

Meister, Alfred L., and Charles F. Ritzi.
1958. Effect of chloretone and MS-222 on eastern brook trout. Progressive Fish-Culturist, vol. 20, no. 3, p. 104-110.

MS-222 was considered superior to chloretone as an anesthetic for fishery use. The former had a wider range of practical field concentrations, lesser inhibitory effect on respiration, and was easier and more predictable for use in the field. Both drugs produced more rapid anesthetization when temperatures were increased. Anesthesia with MS-222 was induced within approximately 10 minutes by concentrations ranging from 1:5,000 to 1:15,000 at 37° to 39° F., and 1:5,000 to 1:25,000 at 48° to 51° F. A concentration of 1:1,000 produced respiratory

arrest in brook trout after 5 minutes, but continued exposure for 6 minutes was not fatal.

They observed that 22 to 35 pounds of brook trout, 36 to 86 pounds of salmon or 93 pounds of lake trout could be anesthetized per gram of MS-222.

Moss, D. D., and D. C. Scott.

1964. Respiratory metabolism of fat and lean channel catfish. Progressive Fish-Culturist, vol. 26, no. 1, p. 16-20.

MS-222 was used at the rate of 1 g./3.8 l. to anesthetize channel catfish for measurements of lengths and weights. The fish were placed in a respirometer and recovered from the anesthetic within 1 to 2 minutes. The oxygen consumption of fat fish was greater than that of thin fish at 25° C. At 30° C. the respiratory rates for both groups were similar.

Nelson, P. R.

1953. Use of three anesthetics on juvenile salmon and trout. Progressive Fish-Culturist, vol. 15, no. 2, p. 74.

MS-222, chlorobutanol, and urethane were used as aids in weighing and measuring coho salmon, red salmon, and Dolly Varden trout. MS-222 was used at the rate of 1:12,500 at 12° to 17° C. and effectively anesthetized the fish within several minutes. The concentration was increased slightly for fingerlings. A dosage of 1:10,000 caused 100 percent mortality.

Normandeau, Donald A.

1962. Microhematocrit values for some salmonids reared in New Hampshire. Progressive Fish-Culturist, vol. 24, no. 4, p. 172-176.

A solution containing 1:10,000 of MS-222 was used to anesthetize landlocked salmon, rainbow, brook, lake, and splake trout before collection of microhematocrits. The fish were anesthetized sufficiently within 1 minute. There was a significant relation of mean hematocrits to sampling date, but not to water temperature.

Parkhurst, Z. E., and M. A. Smith.

1957. Various drugs as aids in spawning rainbow trout. Progressive Fish-Culturist, vol. 19, no. 1, p. 39.

MS-222, sodium amytal, methyl pentynol, urethane, and chloretone were used to anesthetize rainbow trout.

A concentration of 264 p.p.m. of MS-222 induced complete anesthesia within 30 to 45 seconds. Longer exposures resulted in some mortality. The fish were in good condition 75 days after treatment. The hatching success of eggs from anesthetized and control fish was similar.

Methyl pentynol at 2,400 p.p.m. was effective within 3.5 minutes, a 0.5-percent solution of urethane in 2 minutes, and 400 p.p.m. of choretone in 1.0 to 1.5 minutes. Sodium amytal, because of its slow action, had no practical value.

The experiments were conducted at a temperature of 43° F.

Phillips, Arthur M., Jr., Henry A. Podoliak, Donald R. Brockway, and Ray R. Vaughn.

1957. The nutrition of trout. Cortland Hatchery Report 26, New York Conservation Department, Fisheries Research Bulletin 21. 93 p.

The absorption of radioactive cobalt was elevated in brook trout which were narcotized with MS-222. It was suggested that there may be an adjustment of the osmotic processes of narcotized fish.

Pickford, Grace E.

1953. A study of the hypophysectomized male killifish, Fundulus heteroclitus (Linn.) Bulletin of the Bingham Oceanographic College, vol. 14, no. 2, p. 5-41.

1957. Methods of hypophysectomy in fishes. Appendix to: The physiology of the pituitary gland of fishes, by Grace E. Pickford and James W. Atz, p. 485-487. New York Zoological Society, New York.

MS-222 is recommended over several other techniques and agents for anesthetizing fish during hypophysectomy. The drug gave excellent results with Fundulus heteroclitus, but suitable strengths must be determined for each species.

Piper, Robert G., and Robert F. Stephens.

1962. A comparative study of the blood of wild and hatchery reared lake trout.

Progressive Fish-Culturist, vol. 24, no. 2, p. 81-84.

Blood samples were collected by heart puncture after anesthetizing the trout in a 1:1,000 solution of MS-222. The hemoglobin levels and erythrocyte counts of the hatchery-reared and wild lake trout were similar.

Pulford, Earl F., and L. M. Woodall.

1963. An operculum marking experiment on juvenile chinook salmon. Research Briefs, Fish Commission of Oregon, vol. 9, no. 1, p. 30-36.

MS-222 was used as an anesthetic during marking of 1.5-inch salmon. There was little mortality of the fish which were maintained under observation for 112 days.

Randall, D. J.

1962. Effect of an anaesthetic on the heart and respiration of telost fish. Nature, vol. 195, no. 4840, p. 506.

Heart and respiratory rates were measured in tench exposed to 25 to 200 mg./l. of MS-222 at 17° C. The heart rate in undisturbed, control fish was 15 to 30 beats per minute. At a concentration of 33 p.p.m. of MS-222, the rate exceeded 50 per minute, and increased at higher dosages. The respiratory rate and amplitude also increased, but in a variable manner.

MS-222 probably acts on the heart via the parasympathetic nervous system since the direct effect of the drug on isolated and perfused hearts of tench, trout and roach decreased the beat frequency. Bilateral sectioning of the vagi of the fish, which were exposed to MS-222, resulted in a reduced heart rate.

Respiratory collapse occurred at 100 to 200 p.p.m. of MS-222.

Robinson, Clay.

1965. Those chasing-rainbows. U.S. Trout News, January-February, p. 5.

The anesthetic action of MS-222 varies according to water temperature and hardness.

Robertson, O. H.

1958. Accelerated development of testis after unilateral gonadectomy, with observations on normal testis of rainbow trout. U.S. Fish and Wildlife Service, Fishery Bulletin, No. 127, vol. 158, p. 9-30.

MS-222 was used to anesthetize fish for gonadectomy and for length and weight measurements. A concentration of 1:20,000 induced adequate anesthesia in 2 to 3 minutes. The fish recovered rapidly, and no harmful effects were observed even after daily use for a number of weeks.

The operational technique included starvation of the fish for 48 hours and initial anesthesia in 1:20,000 of MS-222. The fish were placed on an operating board and then dipped into a 1:25,000 solution of the anesthetic.

The sequence of histological changes in gonadectomized fish and those in which laparotomy only was performed were the same as in normally maturing gonads.

Rodman, Duane T.

1963. Anesthetizing and air-transporting young white sturgeons. Progressive Fish-Culturist, vol. 25, no. 2, p. 71-78.

MS-222, tertiary amyl alcohol, and reduced temperature were employed in air-transporting young sturgeon. Shipments following use of two drugs were not successful. Fish exposed to a 1:40,000 solution of MS-222 survived for 30 to 48 hours but later died. A temperature of 40° F. provided adequate cold sedation which could be maintained during transport for approximately 30 hours with dry ice. The fish were shipped successfully by this method.

Rothlin, E.

1932. M.S. 222 (lösliches Anaesthesin), ein Narkotikum für Kaltblüter. Schweizerische Medizinische Wochenschrift, vol. 62, no. 45, p. 1042-1043.

MS-222 is a third as toxic to cold-blooded animals as novocaine and a tenth as toxic as cocaine. MS-222 was more efficacious in comparison with novocaine, strovain, alypin, tutokain, panthesin, kokaine, barokain, and eukain. A frog was completely anesthetized within 5 to 7 minutes in solutions of 1:1,000 to 1:2,000 of MS-222. Narcosis lasted several hours, and when the animal was placed in fresh water it recovered in 30 to 60 minutes. Studies with homologs of MS-222--allyl, isopropyl, n-butyl ester--gave no better results than the ethyl ester.

Rotmann, Eckhard.
1931. Die Rolle des Ektoderms und Mesoderms bei der Formbildung der Kiemen und Extremitäten von Triton. 1. Operation in Gastrulastadium. W. Roux Archiv fur Entwicklungsmechanik, vol. 124, p. 747-794.

There were no side or after effects of MS-222 on salamanders which were anesthetized by concentrations of 1:3,000 for 1 hour. Repeated anesthetization was not harmful.

Ryder, R. A.
1960. Comparative tagging returns employing three different anesthetics. Canadian Fish Culturist, no. 26, p. 23-25.

Ether, urethane, and MS-222 were used to anesthetize Stizostedion v. vitreum for tagging. After 2 years, approximately twice as many fish tagged with the help of MS-222 had been recovered as those tagged with the help of either of the other anesthetics.

Sakano, Ei-ichi.
1961. Anaesthetizing experiments of chum salmon fry with tricaine methanesulfonate (M.S. 222). Scientific Reports of the Hokkaido Salmon Hatchery, No. 16, p. 103-106.

Sandoz, M.
1920. Recherches experimentales sur les anesthésiques locaux. 1. Preparations et propriétés physiologiques de la tricaïne et de quelques-un de ses dérivés, Bull. Soc. Vaud. Sc. Nat., vol. 53, p. 263-302.

Sandoz Pharmaceuticals
(No date) The toxicity of MS-222 to fish and frogs. Sandoz Pharmaceuticals, Hanover, N.J. (Mimeo) 2 p.

The 30-minute LC_{50} of MS-222 for frogs was 1:160.

A 1:12,200 concentration produced 50-percent mortality of young trout in 15 minutes. The maximal tolerated concentration (LC_1) for trout was 1:15,900 and a 1:25,000 solution induced anesthesia in 99 percent of the individuals (EC_{99}) within 3 to 4 minutes. These data gave a therapeutic index for MS-222 of 1.57.

A 10-percent solution of MS-222 which was stored at room temperature showed no loss in activity after 3 days. After 10 days there was a reduction in activity of about 5 percent. No difference was noted between solutions protected or not protected against light.

Sandoz Pharmaceuticals
(No date) M.S. 222-Sandoz, the anesthetic of choice in work with cold-blooded animals. Sandoz Pharmaceuticals, Hanover, N.J., Technical Bulletin. 10 p.

Concentrations of 0.5 to 1.0 g./gal. anesthetic silver salmon, sockeye salmon, lake trout, brown trout, and largemouth and smallmouth bass within 2 to 4 minutes at 40° to 60° F. A dosage of 0.25 to 1.0 g./gal. is recommended for rainbow trout.

A concentration of 0.14 g./gal. is recommended for tranquilizing bait fish during transport. Levels up to 0.32 g./gal. are effective for various tropical species.

Sato, T.
1930. Beiträge zur Analyse de Wolff'schen Linsen regeneration. Wilh. Roux Archiv für Entwicklungs mechanik d. Oragnismen, vol. 122, p. 451.

Schiffman, R. H., and P. O. Fromm.
1959. Measurement of some physiological parameters in rainbow trout (Salmo gairdnerii). Canadian Journal of Zoology, vol. 37, p. 25-32.

Rainbow trout were anesthetized in 300 p.p.m. of MS-222, placed on their backs and their hearts exposed. Blood samples were collected by cardiac puncture. The samples were divided for measurements of hematocrit, hemoglobin, and erythrocyte count and size. In addition, weights of organs and body water and volumes of blood and plasma were determined.

Schoettger, Richard A., and Arnold M. Julin.
1966. Investigations in Fish Control: 13. Efficacy of MS-222 as an anesthetic on four salmonids. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 19.

Concentrations of MS-222 ranging from 80 to 135 p.p.m. effectively anesthetized rainbow,

brown, brook and lake trout within 3 minutes at 7° to 17° C. The fish were exposed safely to these concentrations for 4 to 12 minutes. Dosages of 50 to 60 p.p.m. induced anesthesia within 15 minutes, and 15 to 30 p.p.m. were effective for sedation. The effective concentrations and exposure times were inversely related to temperature; however, the efficacy of sedating concentrations declined with time at 17° C. The narcotic action of the drug was reversible, provided the fish were removed from the anesthetic prior to the cessation of respiratory activity.

The efficacy of MS-222 was not affected significantly by size of fish or pH of the solution. Anesthetic solutions with hardnesses of 10 p.p.m. were less effective than those containing 35 to 180 p.p.m. total hardness, but the fish treated in soft water recovered sooner.

The exposure tolerances of fish which were repeatedly anesthetized with MS-222 were slightly greater than those which were unexposed previously.

Trout sedated in closed systems at 12° C. reduced their rate of oxygen consumption by 30 percent; the rate was not depressed significantly in open systems.

Serfaty, A., R. Labat, and R. Quillier.
1959. Les réactions cardiaques chez la carpe (Cyprinus carpio) au cours d'une anesthésié prolongée. Hydrobiologia, vol. 13, p. 144-151.

Anesthetizing carp with 100 p.p.m. of MS-222 caused a primary and secondary tachycardia. The first was believed to be correlated with encephalic penetration of the drug, and the second with a reduction of vagal tonus and lack of oxygen. Eventually, auriculoventricular dissociation occurred which was attributed to damage of the intracardiac system.

Shelton, G., and D. J. Randall.
1962. The relationship between heart beat and respiration in teleost fish. Comparative Biochemistry and Physiology, vol. 7, p. 237-250.

The heart and respiratory rates of tench were increased by anesthetizing them in 80 to 200 p.p.m. of MS-222. After 12 minutes in a 200 p.p.m. solution, the fish stopped breathing and the heart rate fell to a level similar to that of unanesthetized individuals. The heart beat and breathing became absolutely synchronized in animals lightly anesthetized in MS-222. The direct effect of MS-222 on isolated hearts was to decrease the beat rate at 15 p.p.m. and decrease beat amplitude above 30 to 60 p.p.m. Since fish have no sympathetic innervation of the heart, the authors suggested the presence of some cardioaccelerator fibers in the vagus nerve.

Smith, Lloyd L., Jr., Robert H. Kramer, and J. Cameron MacLeod.
1965. Effects of pulpwood fibers on fathead minnows and walleye fingerlings. Journal, Water Pollution Control Federation, vol. 37, no. 1, p. 130-140.

A concentration of 1,000 p.p.m. of MS-222 rapidly anesthetized fathead minnows for the measurement of hematocrits. The hematocrits of treated and control fish were not significantly different. The quantity of blood obtained from anesthetized individuals was 17 percent less than controls and probably indicates a reduced rate of circulation.

Smith, Lynwood S., and Gordon R. Bell.
1964. A technique for prolonged blood sampling in free-swimming salmon. Journal of the Fisheries Research Board of Canada, vol. 21, no. 4, p. 711-717.

The dorsal aortas of pink and sockeye salmon were cannulated to permit the sampling of blood over extended periods. The fish were anesthetized during the operation by irrigating the gills in a 1:15,000 solution of MS-222 using a pump recycling system. The cannula was attached to a length of polyethylene tubing which extended dorsal from the roof of the mouth to above the snout.

Snieszko, S. F.
1960. Microhematocrit as a tool in fishery research and management. U.S. Fish and Wildlife Service, Special Scientific Report--Fisheries No. 341. 15 p.

A technique for measuring the hematocrit of fish was described. The fish were anesthetized for approximately 1 minute in a 1:2,000 solution of MS-222.

Steinbrecht, Karl.

1957. Narkose von Fischen. Die Aquarien- und Terrarien-Zeitschrfit, vol. 10, no. 11, p. 305-306.

There were no abnormalities of offspring from guppies which were frequently anesthetized in a 1:2,000 solution of MS-222 following fertilization.

Steucke, Erwin, W., Jr., and Charles R. Atherton.

1965. Use of microhematocrit values to sex largemouth bass. Progressive Fish-Culturist, vol. 27, no. 2, p. 87-90.

MS-222 at 1:3,000 was used to anesthetize largemouth bass. They were narcotized in about 2 minutes.

Thompson, R. B.

1959. Tricaine methanesulfonate (M.S. 222) in transport of cutthroat trout. Progressive Fish-Culturist, vol. 21, no. 2, p. 96.

Young cutthroat trout were transported successfully in plastic bags which contained oxygen and a 1:40,000 solution of MS-222. The temperature was reduced by packing the bags in ice.

One-quart, plastic food boxes were tested in place of plastic bags. Approximately 500 individuals were added to each box which was half filled with the 1:40,000 solution of anesthetic. Oxygen was not used in these tests, but the containers were packed in ice. The fish were released after 3 hours, and only 19 out of 2,000 failed to recover from anesthesia.

Villwock, W.

1958. Narkose bei Fischen. Aquarien-Terrarien-Zeitschrift, vol. 11, p. 28.

Walker, Charles R., and Richard A. Schoettger.

1966. Investigations in Fish Control: 15. Residues of MS-222 in four salmonids following anesthesia. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 21.

Residues of MS-222 were measured in rainbow, brown, brook, and lake trout. The fish were anesthetized to medullary collapse in concentrations of 80 to 135 p.p.m., depending on species and temperature. Residues ranged from 6 to 72 p.p.m. in the muscle tissues of fish at the time of medullary collapse. The levels in fish which had recovered in freshwater at 12° C. declined rapidly within 3 hours and approached background values after 6 to 9 hours. A slower dissipation of residues occurred in tests at 7° and 17° C. The total residue including background did not exceed 5 p.p.m. at the end of 9 hours, or 3 p.p.m. after 24 hours for all species at all temperatures.

Residues of MS-222 in the blood, liver, and kidney of rainbow trout declined in a pattern similar to that for muscle.

1966. Investigations in Fish Control: 14. Method for determining MS-222 residues in fish. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 20.

The Bratton-Marshall method is sensitive for primary aromatic amines such as sulfa drugs. It was modified to detect MS-222.

Selected tissues from anesthetized trout were processed to extract the drug. The recovery of known amounts in muscle, blood, kidney, and liver ranged from 90 to 112 percent. The concentrations of interferring amines were evaluated.

The acute oral LD_{50} of MS-222 to laboratory rats is between 5 and 10 g./k. This indicates a relatively low toxicity of MS-222 to mammals.

Watson, John E.

1961. Tricaine methanesulfonate as an anesthetic for herring. Progressive Fish-Culturist, vol. 23, no. 4, p. 174.

MS-222 was tested in sea water as an anesthetic for herring. The fish were anesthetized within 8 minutes by a solution of 1:20,000 at 8° C. They recovered in approximately 8 minutes after removal to fresh seawater. Their prolonged exposure to MS-222 for 3 to 4 minutes after complete anesthesia was usually lethal.

Webb, Robert T.

1954. Tricaine methanesulfonate (M.S. 222) as an anesthetic for some common pond fishes. Unpublished thesis. Alabama Polytechnic Institute, Auburn.

Webb, Robert T.
1958. Distribution of bluegill treated with tricaine methanesulfonate (M.S. 222). Progressive Fish-Culturist, vol. 20, no. 2, p. 69-72.

Tests were conducted to determine whether the application of MS-222 would increase the number or pounds of bluegills which could be carried in a distribution truck. A concentration of 0.1 g./gal. appeared to be the most promising. The results of the tests were inconclusive; successful tests could not be duplicated; and at times the control fish hauled as well or better than the drugged ones.

Witschi, E.
1927. Testis grafting in tadpoles of Rana temporaria L. and its bearing on the hormone theory of sex determination. Journal of Experimental Zoology, vol. 47, p. 269.

Wood, E. M.
1956. Urethane as a carcinogen. Progressive Fish-Culturist, vol. 18, no. 3, p. 135-136.

Urethane induces lung tumors in mice which develop whether the drug is administered by injection, in drinking water, by nasal instillation, or from painting the skin.

An editorial comment accompanying this report indicated that MS-222 might be a suitable substitute for urethane.