

# TRICAINE-S

Brand of Tricaine Methanesulfonate  
For Anesthesia and Tranquilization of Fishes and  
Other cold-blooded Animals  
KEEP TIGHTLY CLOSED

USE ONLY FRESH SOLUTION

**CAUTION:** It is imperative to read accompanying descriptive literature before using this drug.

Store at room temperature  
(Approximately 25°C (77°F))

KEEP OUT OF REACH OF CHILDREN

TRICAINE-S is intended for the temporary immobilization of fish, amphibians, and other aquatic, cold-blooded animals. It has long been recognized as a valuable tool for the proper handling of these animals during manual spawning (fish stripping), weighing, measuring, marking, surgical operations, transport, photography, and research.

### WARNINGS

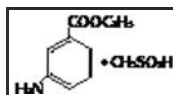
Do not use within 21 days of harvesting fish for food.

When used in food fish, use should be restricted to *Ictaluridae*, *Salmonidae*, *Esocidae*, and *Percidae* and water temperature should not exceed 10°C (50°F).

In other fish and other cold-blooded animals (poikilotherms), TRICAINE-S should be limited to hatchery or laboratory use.

### CHEMISTRY

TRICAINE-S is the methanesulfonate of meta-amino benzoic acid ethylester, or simply ethyl *m*-amino benzoate. It is thus an isomer of benzocaine having the formula  $C_9H_{11}O_2N + CH_3SO_3H$  and the following structure:



TRICAINE-S is a fine white crystalline powder. Its molecular weight is 261.3. Soluble to 11%, it forms clear, colorless, acid solutions in water.

### TOXICOLOGY

Comparative toxicologic studies carried out on fish and frogs gave the following results: FISH TOXICITY STUDIES -The toxicity of TRICAINE-S was measured by standard methods in laboratory bioassays with rainbow trout, brown trout, brook trout, lake trout, northern pike, channel catfish, bluegill, largemouth bass, and walleye. The 24, 48 and 96 hour LC<sub>50</sub> (lethal concentration for 50 percent of the animals) values for trout ranged from 52 to 31 mg/liter; for northern pike, from 56 to 48 mg/liter; for catfish, from 66 to 50 mg/liter; for bluegill and largemouth bass, from 61 to 39 mg/liter; and for walleye, the values were 49 to 46 mg/liter.

**Safety index:** The safety indices for TRICAINE-S refer to the margin between concentrations which cause anesthesia and mortality. They are expressed by the quotient of the lethal concentration for 50 percent of the fish (LC<sub>50</sub>) and the effective concentration for 50 percent of the fish (EC<sub>50</sub>).

#### Safety Indices for Rainbow Trout and Channel Catfish at 12°C (54°F).

Species	Exposure (min.)	LC <sub>50</sub> (mg/liter)	EC <sub>50</sub> (mg/liter)	Index
Rainbow trout <sup>1</sup>	15	65	32	2.0
"	30	57	32	1.8
"	60	56	29	1.9
Channel catfish <sup>2</sup>	15	139	47	3.0
"	30	118	45	2.6
"	60	110	46	2.4

FROG TOXICITY STUDIES<sup>3</sup> - Frogs were put into various concentrations of TRICAINE-S for 30 minutes and then transferred to tap water in order to determine the LC<sub>50</sub>. The LC<sub>50</sub> was 6.2 percent TRICAINE-S. Therefore, the anesthetic must be used in very high concentration before it is fatal to frogs.

### I. DIRECTIONS FOR USE ON FISH CONCENTRATIONS

TRICAINE-S is effective and safe for the anesthesia of fish when used as directed. Its use is governed by, and can be tailored to, the needs of individual fishery personnel. Sedation and various rates of anesthetization are controlled by the concentration. The versatility of TRICAINE-S is demonstrated by the fact that it has been used in fisheries at levels ranging from 10 to 1,000 mg/liter<sup>3</sup>. The action of the anesthetic is slowed at cooler temperatures, in extremely soft water (approximately 10 mg/liter of CaCO<sub>3</sub>, or less), and in larger fish<sup>4</sup>. Also, efficacy may vary with species<sup>4</sup>. Thus, it is imperative that preliminary tests of anesthetic solutions be made against small numbers of fish to determine the desired rate of anesthesia and exposure times for the specific lots of fish under prevailing conditions.

The following tables may be used as guidelines in selecting concentrations of TRICAINE-S for the anesthetization of various fishes:

**Table 1 - Concentrations Required for Rapid Anesthesia**  
(Induction time less than 2-5 minutes;  
used in spawning, marking, measuring, and some surgical operations)

Fish	Temperature	Concentration (mg/liter)	Max. tolerated exposure time* (min.)	Recovery time in fresh water (min.)
<i>Salmonidae</i> <sup>4</sup> (Pacific and Atlantic salmon; trout; charrs; etc.)	7 - 17°C (45 - 63°F)	80 - 135	4 - 12	3 - 19
<i>Esocidae</i> <sup>5</sup> (Northern Pike; muskellunge)	8 - 12°C (46 - 54°F)	150	8 - 28	8 - 31

**Table 1 - Concentrations Required for Rapid Anesthesia continued**

Fish	Temperature	Concentration (mg/liter)	Max. tolerated exposure time* (min.)	Recovery time in fresh water (min.)
<i>Cyprinidae</i> <sup>3</sup> (Carp; goldfish)	16°C (61°F)	150 - 200		
<i>Ictaluridae</i> <sup>2</sup> (Channel catfish)	7 - 27°C (45 - 81°F)	140 - 270	4 - 11	3 - 24
<i>Centrarchidae</i> <sup>4</sup> (Bluegill; largemouth bass)	10 - 27°C (50 - 81°F)	260 - 330	3 - 5	7 - 11
<i>Percidae</i> <sup>3</sup> (Walleye)	10 - 16°C (50 - 61°F)	100 - 120	7 - 18	5 - 40
<i>Pet and Tropical</i> <sup>1</sup> Live-bearers	24 - 27°C (75 - 81°F)	85	12 hrs.	
Egg layers	24 - 27°C (75 - 81°F)	75	12 hrs.	

\* Maximum tolerated exposure time (in minutes) of fish to TRICAINE-S solution.

**Table 2 - Concentrations Required for Moderately Rapid Anesthesia**

(Induction time less than 15-20 minutes; used in surgical operations and in spawning and marking where longer exposures are more important than rapid immobilization)

Fish	Temperature	Concentration (mg/liter)	Maximum tolerated exposure time* (min.)	Recovery time in fresh water (min.)
<i>Salmonidae</i> <sup>4</sup> (Pacific and Atlantic salmon; trout; charrs; etc.)	7 - 17°C (45 - 63°F)	50 - 60	30 or >	2 - 20
<i>Ictaluridae</i> <sup>2</sup> (Channel catfish)	7 - 27°C (45 - 81°F)	70	30 or >	1 - 10

\* Maximum tolerated exposure time (in minutes) of fish to TRICAINE-S solution.

**Table 3 - Concentrations Required for Sedation**

(Induction within 15 minutes; used in fish transport)

Fish	Temperature	Concentration (mg/liter)	Maintenance of Sedation (hr.)
<i>Salmonidae</i> <sup>4</sup> (Pacific and Atlantic salmon; trout; charrs; etc.)	7 - 17°C (45 - 63°F)	15 - 30	6
<i>Esocidae</i> <sup>5</sup> (Chain pickerel)		40	
<i>Ictaluridae</i> <sup>2</sup> (Channel catfish)	7 - 27°C (45 - 81°F)	20 - 40	6
<i>Centrarchidae</i> <sup>2</sup> (Bluegills)		25	8 - 13
<i>Pet and Tropical</i> <sup>1</sup> [Bettas, Piranhas, etc. (uncrowded)]	24 - 27°C (75 - 81°F)	66	48
Goldfish	24 - 27°C (75 - 81°F)	37	48

**IMPORTANT:** Since, in many cases, relatively rapid rates of anesthesia can be achieved only by exceeding the lethal concentration of TRICAINE-S, it is necessary to return anesthetized fish to fresh water before they are overexposed. Excessive exposures are avoided by observing the following sensory and motor responses of the fish which characterize progressively deeper levels of anesthesia:

Sedation - Decreased reactivity to visual and vibrational stimuli; opercular activity reduced.

Total loss of equilibrium - Fish turns over; locomotion ceases; fish swims or extends fins in response to pressure on caudal fin or peduncle.

Total loss of reflex - No response to pressure on caudal fin or peduncle; opercular rate slow and erratic.

Medullary collapse - Opercular activity ceases.

Laboratory and field investigations<sup>3,9</sup> have shown that the action of TRICAINE-S is readily reversed when the fish are transferred to fresh water before opercular activity ceases. Additional exposure following medullary collapse may result in mortality. A rough estimate of the safe total exposure can be made by multiplying the time required for anesthesia by a factor of 2 or 3.

### WATER

Since TRICAINE-S is very soluble (1:9) in water, it dissolves with equal readiness in spring water, tap water, or sea-water. Do not use distilled or deionized water, or water containing chlorine, heavy metals (copper, zinc, etc.), or other toxic contaminants. The anesthetic solution should be well oxygenated, and its temperature should be similar to that of the water from which the fish are taken. In the field, many water quality problems are eliminated by using natural water to which the fish are acclimated, provided the water does not possess high chemical or biologic oxygen demand.

### METHODS OF APPLICATION

**1. General anesthesia:** For most situations where rapid or moderately rapid anesthesia is required, TRICAINE-S may be applied in a bath, i.e., the fish are immersed in the anesthetic solution. Containers may be of glass, plastic, steel, aluminum, or other suitable material. However, do not use galvanized or brass containers unless treated or sealed to prevent dissolution of zinc. Size of container is determined by individual needs, but the fish should not be overcrowded. Discard anesthetic solutions when a loss in potency is noted, or when the solutions become fouled with mucus or excrement.

**2. For surgery and certain physiologic studies,** the fish may be anesthetized to loss of reflex, removed from the anesthetic, and then positioned so that the gills are bathed in a sedating concentration of TRICAINE-S. Some investigators have developed flowing, recirculating systems for bathing the gills with anesthetic during surgery.

Large fishes such as sharks and rays are anesthetized within minutes by spraying the gills with a 1 g/liter solution of TRICAINE-S.<sup>10</sup> The application is made by means of a water pistol, bulb syringe, hand pump, etc.

**3. Transport** - TRICAINE-S has been used to sedate fish during transport. It is more successful in cold than in warm water, and it is instrumental in reducing injuries because of hyperactivity. Fish are usually transported by means of distribution units (tank trucks), or by air in plastic bags.<sup>11,12</sup> In either case, the fish should be fasted before-hand to reduce

metabolic wastes. Also, some workers suggest pre-transport sedation for several hours to lower metabolism. With distribution units, the fish may be fasted and sedated prior to loading. The anesthetic solution is prepared in the distribution unit and oxygenated. Then, the fish are added and temperature acclimated.

In air shipments, the anesthetic solution is placed in a suitable plastic bag, the sedated fish are added, the bag inflated with oxygen, tied securely, and placed in a second bag. This bag is also tied, and then placed on ice in an insulated container<sup>13</sup>. A modification of this method involves complete anesthesia of the fish, and placing them in water bags which contain no anesthetic. In any case, upon arrival, the fish should be acclimated slowly to new environmental temperatures.

#### PREPARATION OF TRICAINES SOLUTIONS

Prior to use, TRICAINES may be weighed out into amounts which are convenient for the volume of water to be used. A handy unit is 2 g. since this quantity in 5 gallons of water yields a concentration of about 100 mg/liter. For rough approximations, one level teaspoonful contains 2.0 to 2.5 g. Thus, a level teaspoonful of anesthetic in 5 gallons gives a concentration of about 120 mg/liter.

To convert mg/liter into g/gal.: multiply number of mg. by 0.00378.

e.g. 80 mg/liter = 80 x 0.00378 = 0.302 g/gal.

To convert mg/liter into a ratio of TRICAINES to water: divide 1,000,000 by the number of mg.

e.g. 80 mg/liter = 1,000,000 ÷ 80 = 1:12,500

#### LIMITATIONS IN USE

Since TRICAINES is taken up into the blood of fish, residues of the drug may occur in edible tissues. However, the residues dissipate rapidly after the fish are placed in fresh water<sup>14</sup>. Thus, treated fish which may be used for food must be held in fresh water above 10°C (50°F) for a period of 21 days.

Withdrawal in fresh water is unnecessary for non-food fishes such as goldfish, bait fish, and ornamentals. Also, withdrawal is unnecessary for sublegal sizes of the following species of fish because they are not used as food immediately following anesthesia (Table 4).

**Table 4 - Sublegal Sizes of Fish Species Not Used as Food Immediately after Anesthesia<sup>15</sup>**

Species	Size (in.)	Species	Size (in.)
Pink salmon	6	Lake trout	5
Chum salmon	6	Splake trout	6
Coho salmon	6	Grayling	6
Sockeye salmon	6	Northern pike	12
Chinook salmon	6	Muskellunge	12
Cutthroat trout	6	Channel catfish	6
Steelhead trout	8	Flathead catfish	6
Rainbow trout	6	Bluegill	3
Atlantic salmon	10	Redear sunfish	3
Brown trout	6	Smallmouth bass	5
Brook trout	6	Largemouth bass	5
		Walleye	6

#### PRECAUTIONS

1. Avoid inhaling TRICAINES or getting it into the eyes.
2. Always conduct preliminary tests with TRICAINES to determine desired rates of anesthesia and optimal length of exposure.
3. Do not overexpose fish to lethal levels of TRICAINES.
4. Do not anesthetize more fish than can be handled effectively.
5. Do not contaminate eggs or sperm with TRICAINES when stripping fish.
6. Do not use water containing chlorine, or other toxic agents.
7. Insure adequate oxygen in anesthetic solution.
8. Discard anesthetic solutions when fouled with mucus or metabolic wastes.
9. Do not discard TRICAINES solutions into water supplies or natural waters.
10. Store TRICAINES solutions in a cool place away from light.\*
11. Discard stock solutions of TRICAINES after several days.\*
12. Treated fish destined for food must be held in fresh water above 10°C (50°F) for 21 days before use.

\* The color of TRICAINES solutions may change rapidly to yellow or brown when exposed to light. This does not affect activity in any significant way. However, for best results, use freshly prepared solutions. A 10 percent solution stored at room temperature shows no significant loss of potency after three days, but after 10 days, a brownish color and an activity decrease of 5 percent is observed.

#### II. GUIDELINES FOR USE ON AMPHIBIANS

**Table 5 - Effects of Varying Concentrations of TRICAINES on Salamanders**

Salamander	Concentration*	Duration of Anesthesia*	Remarks
EMBRYOS <i>Ambystoma opacum</i>	1:10,000 (3b)	2 days	No adverse effects
	1:3,000 (3c)	to 30 min.	
LARVAE <i>Ambystoma opacum</i>	1:10,000 (3b)	2 days	No adverse effects.
	1:12,000 (3f)	10-15 min.	
	1:20,000 (3f)	10-15 min.	
	1:3,000 (3c)	to 30 min.	
ADULTS	1:1,000 (3b)	few min.	No adverse effects.
Newts	1:3,000 (3b)	3 days	No adverse effects.
	1:1,000 (3b)	few min.	
<i>Triturus</i> sp.	1:10,000 (3b)	2 days	No adverse effects.
	1:1,000 (3k)	20 min.	
<i>Triturus uridescens</i>	1:3,000 (3g)	1 hour	"
Mole salamanders <i>Ambystoma opacum</i>	1:3,000 (3c)	to 30 min.	No adverse effects.
<i>Ambystoma tigrinum</i>	1:2,000 (3j)	15 - 30 min.	"
<i>Ambystoma punctatum</i>	1:2,000 (3j)	15 - 30 min.	"
Mud-puppy <i>Necturus maculosus</i>	1:1,500 (3i)	to 6 hours	**

\*\* Maintenance dose, 0.1 of induction concentration. At exposure to induction concentration for more than 20-30 minutes, renal circulation becomes sluggish or stops

\* When an individual of any of the species listed is exposed at the designated concentration, the data available suggests that the animal may be safely maintained under anesthesia for the time noted. Prolonging exposure to the anesthetic beyond the time indicated may cause deaths. See PRECAUTIONS.

**Table 6 - Effects of Varying Concentrations of TRICAINES on Frogs**

Frog	Concentration*	Duration of Anesthesia	Remarks:
EMBRYOS	1:1,000 (3b)	few min.	No adverse effects.
	1:10,000 (3b)	2 days	
	1:15,000 (3h)	3 days	
TADPOLES	1:1,000 (3j)	30 min.	No adverse effects.
	1:3,000 (3f)	10 - 15 min.	
	1:10,000 (3b)	2 days	
	1:15,000 (3h)	3 days	
<i>Rana</i> sp.	1:5,000 (3k)	5 hours	No adverse effects.
<i>Rana pipiens</i>	1:1,000 (3j)	15-30 min.	
ADULTS	1:3,333 (3a)	2 min.	No adverse effects.
	variable (3d)	1 hour	
	1:1,000 (3e)	30min.	
	Leopard frog <i>Rana pipiens</i>	1:3,000 (3c)	
Eastern wood frog <i>Rana sylvatica</i>	1:8,000 (3l)	5 - 10 min.	Only slightly under anesthesia

\* When an individual of any of the species listed is exposed at the designated concentration, the data available suggests that the animal may be safely maintained under anesthesia for the time noted.

Prolonging exposure to the anesthetic beyond the time indicated may cause deaths. See PRECAUTIONS.

#### AVAILABILITY OF TRICAINES

Bottles of 1 kilogram, 100 grams, 10 grams, 5 grams and 1 gram.

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